DIVERSIFICATION PROCESSES IN AN ISLAND RADIATION OF SHREWS

BY

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ABSTRACT

Southeast Asian mammals are known for their remarkable levels of diversity and endemism. However, few explicit tests of the mechanisms that may promote or inhibit speciation have been conducted on regional clades. I use phylogenetic estimates and tree shape analyses to explore the tempo and mode of diversification in Southeast Asian shrews (Soricomorpha: *Crocidura*), and to consider a set of geological, climatic, and ecological forces that my have shaped current patterns of diversity. I find no association of diversification rates with Pleistocene sea-level fluctuations or volcanic uplift that was concentrated during the Miocene and Pliocene. However, sea-level fluctuations appear to have been a factor in the generation of phylogeographic diversity in the Philippines. In general, *Crocidura* appears to have diversified at a consistent tempo and usually in allopatry. A lack of ecological innovation may have limited the extent of diversification in the Philippines, but perhaps not on Sulawesi.

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INTRODUCTION

Southeast Asian biodiversity has long piqued the interest of biogeographers and systematists (e.g., Wallace 1860). The region's diversity is staggering, housing approximately 20% of global mammal diversity (Corbet and Hill 1992). However, despite the extensive interest, relatively few mechanistic explanations have been proposed for how the region became so diverse. For instance, biologists studying patterns of diversity in particular clades often allude to the complexity of Southeast Asia's geological history, sea-level fluctuations, and climate change as mechanisms of speciation, but few explicit tests have been undertaken. Herein I explore the phylogenetic and phylogeographic history of a group of shrews inhabiting many islands in Southeast Asia. My goal is to articulate and test explicit hypotheses that potentially explain current patterns of diversity. Over the course of this exercise, the phylogenetic and taxonomic diversity of shrews, and their fundamental biogeographic history are illuminated. Specifically, I test for elevated diversification rates associated with Pleistocene sea-level fluctuations and the complex set of geological processes that greatly altered the distribution of islands during the Miocene and Pliocene (Chapter 1). The potential role of sea-level fluctuations in shaping intraspecific diversity is further considered in Chapter 2 and the importance of long-distance, over-water island colonization is evaluated in Chapter 3. Finally, I close by exploring the potential roles of ecological opportunity and competitive exclusion in constraining diversification in the Philippine archipelago (Chapter 4).

CHAPTER 1

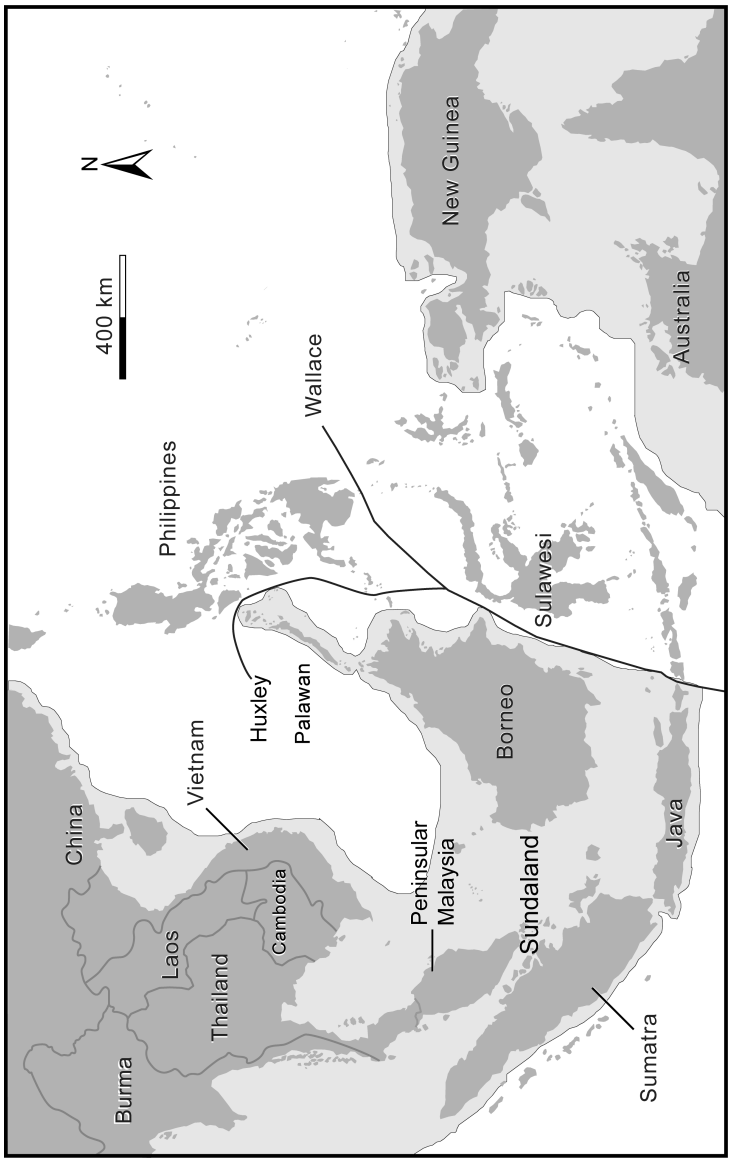
Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews

A decline in the net rate of diversification through time is commonly inferred from molecular phylogenies (Kozak et al. 2006; McPeek 2008; Price 2008; Rabosky and Lovette 2008). This pattern is frequently characterized as evidence for density-dependent diversification, which supports the concept of a correlation between speciation rates and ecological opportunity (Seehausen 2007). Hence, density-dependent diversification is a central tenet of the 'ecological theory' of adaptive radiation and may apply broadly to non-adaptive radiations as well (McPeek 2008; Schluter 2000; Seehausen 2007). However, Phillimore and Price (2008) argued that the commonness of declining rates of diversification is partially due to the stochastic nature of birth-death processes. They demonstrated that clades that speciate rapidly early in their history tend to have many extant species, and thus are subject to phylogenetic study. Whatever the cause, most studies investigating the tempo of diversification examine continental radiations and many have inferred the putative density-dependent pattern (McPeek 2008; Phillimore and Price 2008; Price 2008). Although island faunas have been the focus of intensive study by evolutionary biologists, it remains an open question whether declining rates of diversification is the norm in island archipelagos, where there are enormous opportunities for allopatric diversification (Arbogast et al. 2006; Brown and Guttman 2002; Evans et al. 2003a; Filardi and Moyle 2005; Grant et al. 2000; Steppan et al. 2003).

The archipelagos of Southeast Asia represent the largest complex of islands in the world (Fig. 1.1), and they house a substantial proportion of global biodiversity (Mittermeier et al. 2004). The region is an aggregate of three globally significant hotspots divided by sharp, yet porous biogeographic boundaries (Evans et al. 2003a; Schmitt et al. 1995; Wallace 1860). Dynamic geological and climatic histories have combined to generate a matrix of islands in which the spatial distribution of terrestrial habitats has been altered extensively through time (Bird et al. 2005; Hall 1998; Heaney 1985; Voris 2000). The processes of volcanic uplift and repeated sea-level fluctuations represent potential mechanisms promoting evolutionary diversification by providing opportunities for allopatric speciation (Heaney 2000; Jansa et al. 2006; Outlaw and Voelker 2008; Steppan et al. 2003). The two processes are temporally partitioned, with most volcanic uplift taking place before extensive sea-level fluctuations began (Hall 1998, 2002; Haq et al. 1987; Rohling et al. 1998; Zachos et al. 2001). This scenario allows one to test for an impact of each process on diversification by examining temporal variation in the speciation and extinction rates of clades.

Southeast Asian shrews (Soricidae: *Crocidura* [hereafter, "shrews"]) provide an excellent model for testing the impacts of geological and climatic history on phylogenetic diversification. Shrews are broadly distributed across Southeast Asia and probably represent a recent arrival to the region. As species-level diversity in *Crocidura* is highest in Africa, and fossil dates of shrews from the continent are older than those in Eurasia, the group may have originated in Africa (or perhaps western Eurasia) and colonized east Asia relatively recently (Butler 1998; Dubey et al. 2007b, 2008; Hutterer 2005; Storch et al. 1998). Dubey et al. (2007b) estimated the divergence of African from Eurasian *Crocidura* at 5.4–10.7 mya, thus the entire history of shrew evolution in Southeast Asia

Figure 1.1. Map of Southeast Asia showing the extent of modern islands (medium grey) and continental shelves (light grey). Sundaland included the islands of Sumatra, Java, Borneo, and Palawan during Pleistocene glacial maxima. Wallace's Line and Huxley's modification of it are illustrated. Shrews (*Crocidura*) occur widely across the Sunda Shelf and cross Huxley's modification of Wallace's line into the Philippines and Sulawesi.


N
400 km
China
Vietnam
Laos
Thailand
Cambodia
Burma
Peninsular
Malaysia
Sundaland
Sumatra
Java
Borneo
Sulawesi
Philippines
Huxley
Palawan
Wallace
New Guinea
Australia

likely took place during the last 10 million years or so, a period over which we have a good understanding of geological history (Hall 1998, 2002). Shrews are found on all major islands of the Sunda Shelf, and cross Huxley's modification of Wallace's Line into the Philippines and Sulawesi (Fig. 1.1). They are widespread in the Philippines, with nine species currently recognized (Heaney and Ruedi 1994; Hutterer 2007); six species are known from Sulawesi (Ruedi 1995; Ruedi et al. 1998).

We use a multilocus phylogenetic analysis of Southeast Asian shrews to test competing hypotheses of the underlying causes of diversification. Specifically, we test for the monophyly of shrews in the Philippines and on Sulawesi (i.e., single founding colonization event per major landmass or archipelago), for sister relationships between sympatric/syntopic species in the Philippines and Sulawesi (within-island speciation), and for the biogeographical affinities of individual land masses adjacent to the Sunda Shelf. We further use maximum likelihood to fit a series of rate-constant and rate-variable birth-death models to the temporal distribution of speciation events in the phylogeny; we then consider whether the best-fitting models are consistent with the hypotheses of density-dependent diversification (DDD), increased rates of diversification associated with volcanic uplift during the Miocene and Pliocene (MPV), increased rates of diversification associated with Pleistocene sea-level fluctuations (PSL), or a null hypothesis of a constant rate of diversification (CRD; Fig. 1.2).

Methods

Geological History of Southeast Asia

Southeast Asia has a long, complex geological history. The islands of the region are divided into the biogeographic zones of Sundaland (= Sunda Shelf), the oceanic Philippines, and Wallacea (dominated by Sulawesi Island). Sundaland (Malay Peninsula, Borneo, Java, Sumatra, and Palawan) is a complex of large islands currently separated by shallow water, lying south and east of the coasts of Thailand and Cambodia. The area was exposed as dry land repeatedly during Pleistocene glacial maxima (Rohling et al. 1998), thus opportunities for colonization by terrestrial organisms have been frequent, at least throughout the Pleistocene (Bird et al. 2005; Gorog et al. 2004; Heaney 1984; Meijaard and van der Zon 2003; Voris 2000). Sundaland is an important source from which the floras and faunas of the Philippines and Wallacea originated (Corbet and Hill 1992; Dickerson 1928).

Northeast of Sundaland, the Philippines includes >7000 modern islands (Fig. 1.3) that have been converging toward their present location over the last *ca*. 35 million years (Hall 1998, 2002). Most are volcanic in origin, but others are continental fragments that were submerged for long periods of time before emerging as islands (Hall 1998, 2002). The archipelago's fauna is thus derived from over-water colonization (Brown and Guttman 2002; Evans et al. 1999, 2003a; Hall 1998; Heaney 1985). One exception to this pattern is the Palawan group, which was isolated early in its history, but may have had a dry-land connection to Borneo as recently as 165,000 years ago (Hall 1998; Heaney 1984; Voris 2000). The mammalian and avian faunas of Palawan are most similar to those of Borneo (Dickerson 1928; Esselstyn et al. 2004), but the affinities of the herpetofauna are more complex (Brown & Diesmos 2009; Inger 1954). A few studies have examined phylogenetic relationships within clades that span the Borneo–Palawan–Philippines region and several have shown Palawan to have biogeographic relationships

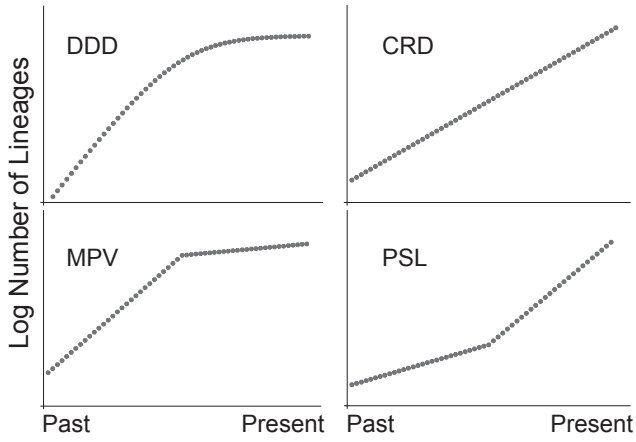


Figure 1.2. Idealized log lineage-through-time plots showing the expected patterns of speciation under hypotheses of density dependent diversification (DDD), a constant rate of diversification (CRD), speciation promoted by Miocene–Pliocene volcanic uplift (MPV), and speciation promoted by Pleistocene sea-level fluctuations (PSL).

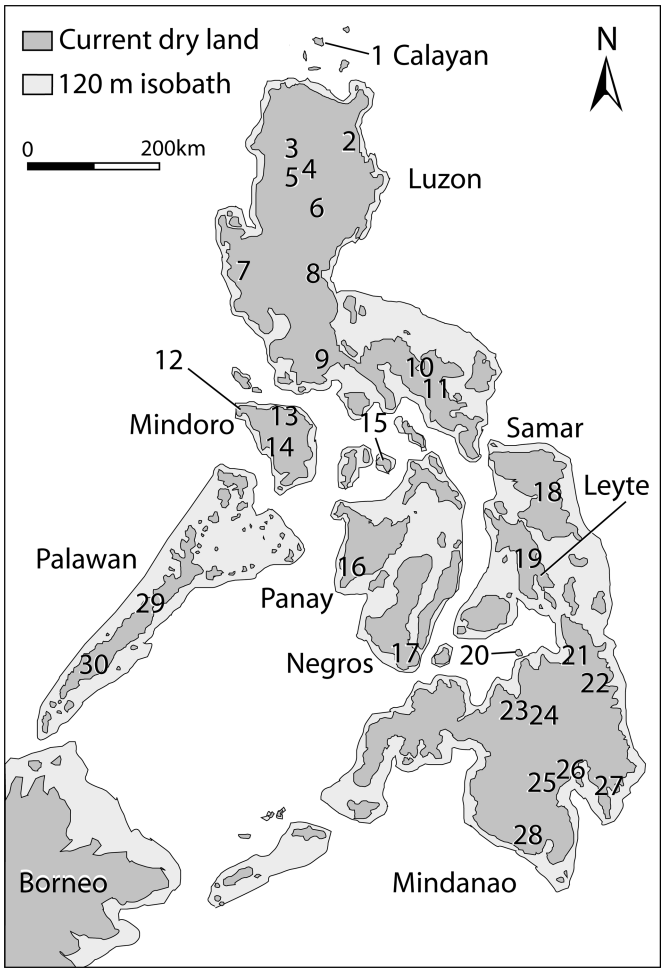


Figure 1.3. Map of the Philippine Islands showing the present distribution of dry land and the extent of dry land during Pleistocene glacial maxima (after Heaney 1985). Numbers show the approximate locations of Philippine sample sites.

with the oceanic Philippines and Sulawesi, often to the exclusion of Borneo (Brown and Guttman 2002; Evans et al. 2003a; McGuire and Kiew 2001).

Lying south of the Philippines and east of Sundaland, the island of Sulawesi probably represents a number of once distinct geological elements that recently coalesced (Evans et al. 2003b; Hall 1998). These former islands correspond today to areas of endemism; each remains a distinctive biogeographic region within Sulawesi (Evans et al. 2003b, 2008). Sulawesi is surrounded by deep water and its individual components probably remained isolated from continental sources throughout their history (Hall 1998, 2002; Voris 2000); thus, the island's biodiversity is also most likely derived from over-water colonization.

Taxon Sampling

We gathered tissue samples from 227 shrews representing >30 species from populations throughout Southeast Asia. Our sampling is densest in the Philippines, where we obtained tissues from seven of nine named species; the two unsampled taxa are *C. grandis*, which is known only from the holotype (Miller 1910), and *C. attenuata* from Batan (a small island lying mid-way between Taiwan and Luzon), which represents an outlying population of a mainland species (Heaney and Ruedi 1994). We include samples of *C. attenuata* from the Asian mainland. All other Philippine taxa are represented, most by multiple specimens from multiple localities; our sampling across geographic space is thorough, with all major Pleistocene island complexes represented (Fig. 1.3). Outside the Philippines, our sampling includes representatives of five species from Sulawesi and five from the Sunda Shelf, including taxa from Sumatra, Java,

Borneo, and Peninsular Malaysia. Additional samples representing seven species from China, Vietnam, Taiwan, and India are included in the analyses.

When analyses were restricted to Cytochrome b (CytB), we further improved our taxonomic sampling with the addition of sequences from GenBank; these provided otherwise unsampled species from Sulawesi (4), the Sunda Shelf (4), Japan and the Ryukyu Islands (2), and the Asian mainland (4; see Appendix I for details). Thus, with the addition of sequences from GenBank, our sampling includes 25 of 31 species known from the region encompassing the Sunda Shelf (including the Malay Peninsula), the Philippines, and Sulawesi (Ruedi 1995) and 34 of 46 species known from the region east of the Thai–Burmese border and south of the Ryukyu Islands (Hutterer 2005; Lunde et al. 2004; Ruedi 1995).

Molecular Genetics

We sequenced the mitochondrial genes CytB and NADH Dehydrogenase Subunit 2 (ND2) along with parts of four flanking tRNAs. We also sequenced three independent nuclear loci. These include the Y-linked Dead Box Intron 14 (DBY14), the autosomal Mast Cell Growth Factor Introns 5–6 (MCGF), and the autosomal exon Apolipoprotein B (ApoB).

We extracted DNA using a non-commercial guanidine thiocyanate method following Esselstyn et al. (2008). The polymerase chain reaction (PCR) was used to amplify target regions of mitochondrial and nuclear DNA. Thermal cycles for PCR followed the general protocol of initial denaturing at 94º for 60 s, followed by 30–40 cycles of denaturing (94º for 30–60 s), annealing (35–60º for 30–60 s), and extension (72º for 30–120 s). Each PCR reaction ended with a final extension at 72º for 5–7 min. We

used several published primers and an array of newly developed, group-specific primers (Table 1.1). Methods of purification and sequencing follow Esselstyn et al. (2008). All sequences were deposited in GenBank under accession numbers FJ813604–FJ814618.

Phylogenetic Analyses

We aligned sequences manually using Se-Al 2.0a11 (Rambaut 1996). The final alignment of the concatenated data set was deposited in TreeBase. No indels were observed in the coding genes (CytB, ND2, ApoB); those found in the introns were short (<10 nucleotides) and alignments were unambiguous. Our phylogenetic inferences relied on parsimony, likelihood, and Bayesian approaches. We used *Suncus murinus* to root all trees because of its position relative to *Crocidura* in recent phylogenetic studies (Dubey et al. 2007b; Ohdachi et al. 2006). A parsimony analysis was conducted in PAUP* 4.0b10 (Swofford 1999) on the concatenated data set. All characters were weighted equally and gaps were treated as missing data. We completed heuristic searches with TBR branch swapping and 500 random addition sequences. One hundred non-parametric bootstraps were completed as measures of clade support.

Bayesian analyses were implemented in MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Sequences were partitioned by codon position for each mitochondrial gene, the four flanking tRNAs were analyzed as a single partition, and each nuclear locus was modeled separately. Appropriate models of sequence evolution for each of the 10 partitions were identified using Akaike's Information Criterion (AIC), as implemented in Modeltest 3.7 (Posada and Buckley 2004; Posada and Crandall 1998). When AIC identified a submodel of the general class of GTR models, the GTR model was used (Table 1.2). Markov Chain Monte Carlo (MCMC) searches of tree space included four

runs with four chains each and were run for 10^7 generations. Trees were sampled every 2000 generations and the first 2001 samples were discarded as burn-in, leaving 3000 post-burnin trees from each run. We sought evidence of convergence among MCMC chains by examining log-likelihood plots in Tracer v1.4 (Rambaut and Drummond 2007). We also examined correlations of split frequencies between runs and cumulative split frequencies in AWTY (Nylander et al. 2008). Separate Bayesian analyses were conducted on CytB, the concatenated nuclear genes, and the entire matrix.

A maximum likelihood analysis was conducted on the expanded CytB data set in RAxMLHPC v7.0 (Stamatakis 2006). We completed 100 iterations of this analysis and selected the best tree among these searches. As our purpose for this inference was to test hypotheses related to rates of net diversification, we wanted as complete taxon sampling as possible with each species represented by a single sequence. We therefore included all available GenBank sequences from east Asian *Crocidura*, but reduced the number of taxa to 50 by limiting each "species" to one sample. For most taxa, this meant a single sequence per named species. However, for several highly variable lineages, we included one representative from each island or each mountain range where populations were inferred to be monophyletic in the Bayesian analysis of the concatenated data. Thus, from the *C. beatus* and *C. grayi* complexes, we included six and five representatives, respectively. We also included one representative of *C. mindorus* from each of the islands it occurs on (Mindoro and Sibuyan) and two highly divergent representatives from each of the mainland taxa, *C. fuliginosa* and *C. wuchihensis*.

Table 1.1. Summary of primers and annealing temperatures used in this study. Annealing temperatures represent the full range used in successful reactions; TD indicates that a "touchdown" protocol was used. Primer names that begin with "Smr" and "Lyt" were designed specifically to amplify mtDNA from populations from the islands of Samar and Leyte, respectively.

Locus	Primer Name	5′	3′	Primers paired with	Annealing Temperatures	Primer Source
ApoB	ApoBf	GCAATCATTTGACTTAAGTG		ApoBr	47–50º	Dubey et al. 2007
	ApoBr	GAGCAACAATATCTGATTGG		ApoBf	47–50º	Dubey et al. 2007
MCGF	MCGF56F	GTTCTCCTCAACATCAAGTCC		MCGF56R	40–55º (TD)	This study
	MCGF56R	GCAATTGCAGAGTTAGGTTCC		MCGF56F	40–55º (TD)	This study
	MCGF56NstF	TGAGAATGGTGYYTGTGTTGAG		MCGF56NstR	43–56º (TD)	This study
	MCGF56NstR	GCCRCCTTCTATTCACCACAG		MCGF56NstF	43–56º (TD)	This study
DBY14	DBY14F	GGGTAGTAAGTTATGTCCC		DBY14R	47–58º (TD)	This study
	DBY14R	GGTTACTCCTGGCTCTATGC		DBY14F	47–58º (TD)	This study
	DBY14NstF1	GTCCCAARATTAACTACTGYTGTTACT		DBY14NstR1	40–60º (TD)	This study
	DBY14NstR1	TATGCTCAGAAATCRCTYCTGGCAA		DBY14NstF1	40–60º (TD)	This study
ND2	Met-1	CTAATAAAGCTTTCGGGCCCATAC		ND2IntR1, LytND2IntR2, Trp-2	49–58º (TD)	Olson et al. 2004
	ND2IntF1	CAGGTTTAATTCTCTTCATGAC		Trp-2	49–56º (TD)	This study
	ND2IntF2	CTATCATAATTGGTGGCTGAGG		Trp-2	44–56º (TD)	This study
	LytND2IntF2	GACATCTATTATAATTGGTGGCTGAGG		Trp-2	35–55º (TD)	This study
	Trp-2	TTCTACTTAAGGCTTTGAAGGC		Met-1, ND2IntF1, ND2IntF2, LytND2IntF2	35–58º (TD)	Olson et al. 2004
	ND2IntR1	AAGTAAGTTTAGGAGGGAGAGG		Met-1	49–56º (TD)	This study
	LytND2IntR1	AGGGAGAGGTTAGGGTTATAG		Met-1	47–55º (TD)	This study
	LytND2IntR2	GACAAGGTAGAGGTAGTTGAAGTA		Met-1	47–55º (TD)	This study
CytB	L14724	CGAAGCTTGATATGAAAAACCATCGTTG		CroCBR, 597R, H15915	40–60º (TD)	Irwin et al. 1991
	29F	ATYCGAAARACYCACCCACT		CroCBF, 597R	45–60º (TD)	This study
	425F	GAGGCCAAATATCATTCTGAGG		1167R, H15915	55–60º (TD)	This study
	CroCBF	TACTTTCAGCTATCCCCTATATCGG		H15915	42–60º (TD)	This study
	SmrCytBNstF2	TCCCAGCACCCTCAAATATCTC		SmrCytBIntR1	50–60º (TD)	This study
	SmrCytBIntF1	ATCGTAGCAGCACTCGCAGGA		SmrCytBExtR	51–60º (TD)	This study
	CroCBR	AATAAGAGATGWACTCCTGCGAG		L14724, 29F	40–60º (TD)	This study
	597R	TTAGAGCCGGTTTCATGTAAG		L14724, 29F	45–60º (TD)	This study
	1167R	CTCCGGTTTACAAGACCAGTR		425F, CroCBF	45–60º (TD)	This study
	H15915	AACTGCAGTCATCTCCGGTTTACAAGAC		L14724, 425F, CroCBF	42–60º (TD)	Irwin et al. 1991
	SmrCytBIntR1	TGTCCGTGTCTGAGTTTAGTCCGGAT		SmrCytBNstF2	50–60º (TD)	This study
	SmrCytBExtR	GACCAGTGTATTARCTATACTACTAAGGC		SmrCytBIntF1	51–60º (TD)	This study

Table 1.2. Summary of models of sequence evolution selected by AIC and used in model-based phylogenetic analyses.

Partition	AIC Model	Model Applied	Number of Characters
Apolipoprotein B	HKY + G	HKY + G	577
Mast Cell Growth Factor Introns 5–6	TVM + G	GTR + G	635
Dead Box Y Intron 14	K81uf + G	GTR + G	485
Cytochrome b, 1st codon position	SYM + I + G	GTR + I + G	380
Cytochrome b, 2nd codon position	HKY + I	HKY + I	380
Cytochrome b, 3rd codon position	GTR + I + G	GTR + I + G	380
NADH 2, 1st codon position	GTR + I + G	GTR + I + G	348
NADH 2, 2nd codon position	TVM + I + G	GTR + I + G	348
NADH 2, 3rd codon position	GTR + I + G	GTR + I + G	348
tRNAs Glu, Thr, Met, Trp	TrN + I + G	GTR + I + G	174

The Role of Inter-Island Colonization

We test several hypotheses related to the origins of Southeast Asian shrew diversity and address the following questions: 1) Are Philippine and Sulawesian shrews each the result of a single founding colonization event? 2) Has within-island speciation occurred in the Philippines or Sulawesi? 3) Do Palawan species (*C. batakorum* and *C. palawanensis*) show a close relationship to Bornean species and/or other taxa from the Sunda Shelf (Esselstyn et al. 2004; Everett 1889; Heaney and Ruedi 1994)? We evaluated each question using Bayesian methods and the Approximately Unbiased (AU) test (Shimodaira 2002). For these questions, the topological constraints consisted of monophyletic lineages including all Philippine species, all oceanic Philippine species, and all Sulawesian species (Hypothesis 1); sister relationships between *C. grayi halconus* and *C. mindorus* from Mindoro Island, between *C. palawanensis* and *C. batakorum* from Palawan Island, and among the several Sulawesian species (Hypothesis 2); *C. palawanensis* and/or *C. batakorum* sister to *C. foetida* or other Sunda Shelf taxa (*C. brunnea*, *C. fuliginosa*, *C. lepidura*, *C. malayana*, *C. maxi*, *C. orientalis*, and *C. paradoxura*; Hypothesis 3). For Hypothesis 3, we considered *C. palawanensis* and *C. batakorum* separately. In these calculations, we used the concatenated and CytB matrices separately. For the Bayesian approach, we took the percentage of 12,000 post-burnin trees consistent with each hypothesis to represent the posterior probability that the hypothesis is true. The AU test comparing the maximum likelihood tree to the maximum likelihood inference under 11 different constraints was implemented using CONSEL v0.1i (Shimodaira & Hasegawa 2001), with per-site likelihood scores generated by RAxMLHPC v7.0 (Stamatakis 2006).

Temporal Patterns of Diversification

We first tested the CytB alignment for the viability of a standard molecular clock. We optimized likelihood scores in PAUP* 4.0b10 with a molecular clock enforced and not enforced on the maximum likelihood CytB topology. We then tested for significantly improved fit to the data with a likelihood ratio test ([LRT] Arbogast et al. 2002; Felsenstein 2004). As the LRT failed to reject a molecular clock, we implemented a strict clock assumption. We calculated two substitution rates derived from Figure 2 of Pesole et al. (1999) to place very approximate divergence date estimates on the ultrametric phylogeny. The rates are one standard deviation greater than and one standard deviation less than the mean mammalian rates for CytB for synonymous and non-synonymous substitutions (Pesole et al. 1999). We then calculated average rates weighted by the ratio of synonymous to non-synonymous substitutions in the *Crocidura* CytB matrix. The resulting substitution rates (one fast and one slow) were then used to place time scales on the ultrametric tree. We then computed the accumulation of lineages through time (LTT) in GENIE v3.0 (Pybus and Rambaut 2002).

We used a maximum likelihood, model-fitting approach to test for variation in diversification rates (Rabosky 2006b). We chose this method over others because it is the only available technique capable of detecting increases in diversification rates through time, it has the potential to distinguish gradual from instantaneous changes in rates, and it outperforms other methods when extinction is present (Rabosky 2006b). We fit a variety of rate-constant and rate-variable versions of pure birth and birth-death models to the distribution of splitting events in the phylogeny using the R package, LASER 2.0 (Rabosky 2006a). The likelihood of each model was maximized over

parameter space and model fit was measured using AIC; we compared the fit of the best rate-constant model to the fit of the best rate-variable model using the statistic, ΔAIC, as:

$$\Delta AIC = AIC_{rc} - AIC_{rv},$$

where AIC_{rc} is the AIC score of the best fitting rate-constant model and AIC_{rv} is the AIC score of the best fitting rate-variable model (Rabosky 2006b). ΔAIC is positive when a rate-variable model provides better fit than the rate-constant models and negative when a rate-constant model provides the best fit. Null distributions of ΔAIC scores were generated by fitting the same candidate models to 5000 trees simulated under the hypothesis of a constant-rate, pure-birth process. We accounted for uncertainty associated with incomplete taxon sampling by pruning randomly selected taxa from the simulated phylogenies before fitting the birth-death models. Simulated trees held the same diversity (total number of taxa and number of missing taxa) as the empirical phylogenies. ΔAIC scores from the observed phylogeny were then compared to these null distributions to determine significance. Type I error rates can be high in model fitting exercises when a lower AIC score is the sole criterion used to evaluate fit; generation of null distributions is therefore necessary to maintain Type I error rates near 0.05 (Rabosky 2006b).

We considered whether the results of these model-fitting analyses were consistent with the null hypothesis (CRD) or its alternatives (DDD, MPV, and PSL; Fig. 1.2). These hypotheses incorporate the following predictions: If shrews have diversified in a manner consistent with the null hypothesis (CRD), then either the rate-constant Yule

model or the rate-constant birth-death model should provide the best fit. If Pleistocene sea-level fluctuations resulted in elevated speciation rates (PSL), we expect to observe an instantaneous shift from a slow rate to a fast rate of diversification, with either the Yule-2-Rate or Rate-Variable Birth-Death (RVBD) model providing the best fit. If either the MPV or DDD hypothesis is operating, we should see a decline in rates through time. MPV predicts an instantaneous shift (Yule-2-Rate or RVBD), whereas DDD predicts a gradual decline (logistic or exponential density-dependent models). In principle, MPV and DDD are distinguishable; in practice, differentiating between them will be difficult. Testing the MPV hypothesis requires the assumption that shrews arrived in Southeast Asia well before the Pliocene–Pleistocene boundary (1.8 mya). This assumption is reasonable, considering that Dubey et al. (2008) estimated the age of the earliest ingroup node in our *Crocidura* phylogeny at 6 mya and the origin of the primary clade that invaded Sundaland and the Philippines at 4.4 mya, suggesting that *Crocidura* colonized the islands of Southeast Asia at least 2 my before the beginning of the Pleistocene (1.8 mya). Because we calibrate the phylogeny to two potential time scales, either or both of which could be grossly incorrect, we allow shift times to vary in the models, and it is the relative position of fast and slow rates that will allow us to distinguish among hypotheses.

To evaluate statistical power, we simulated 1000 trees using a pure birth model with two rates of speciation, one fast and one slow (Python code provided by Mark T. Holder). These simulations were intended to mimic a shift in diversification rates at or near the Pliocene–Pleistocene boundary. We simulated data where diversification rates shifted to faster and slower rates by 1.5-, 2-, and 4-fold at three evenly spaced points in time. Rates shifted when the number of taxa in the growing tree was 0.25, 0.5, and 0.75 of the final number. Simulated trees contained the same diversity as the empirical

phylogenies, with randomly selected taxa removed to accommodate uncertainty associated with incomplete taxon sampling. We fit the same candidate models to these simulated data, and used the distribution of ΔAIC scores to infer the probability of rejecting the null hypothesis (CRD). The proportion of ΔAIC scores with higher values than the critical value in the null simulation was taken as the power to reject CRD under these scenarios. Because we were concerned that patterns of diversification might differ among individual clades within the entire data set, all of these analyses were conducted separately for the entire phylogeny (49 species sampled and 12 missing) and a well-sampled, monophyletic group distributed across the Philippines, Sulawesi, and the Sunda Shelf (23 species sampled and 6 missing).

Results

Phylogeny Estimation

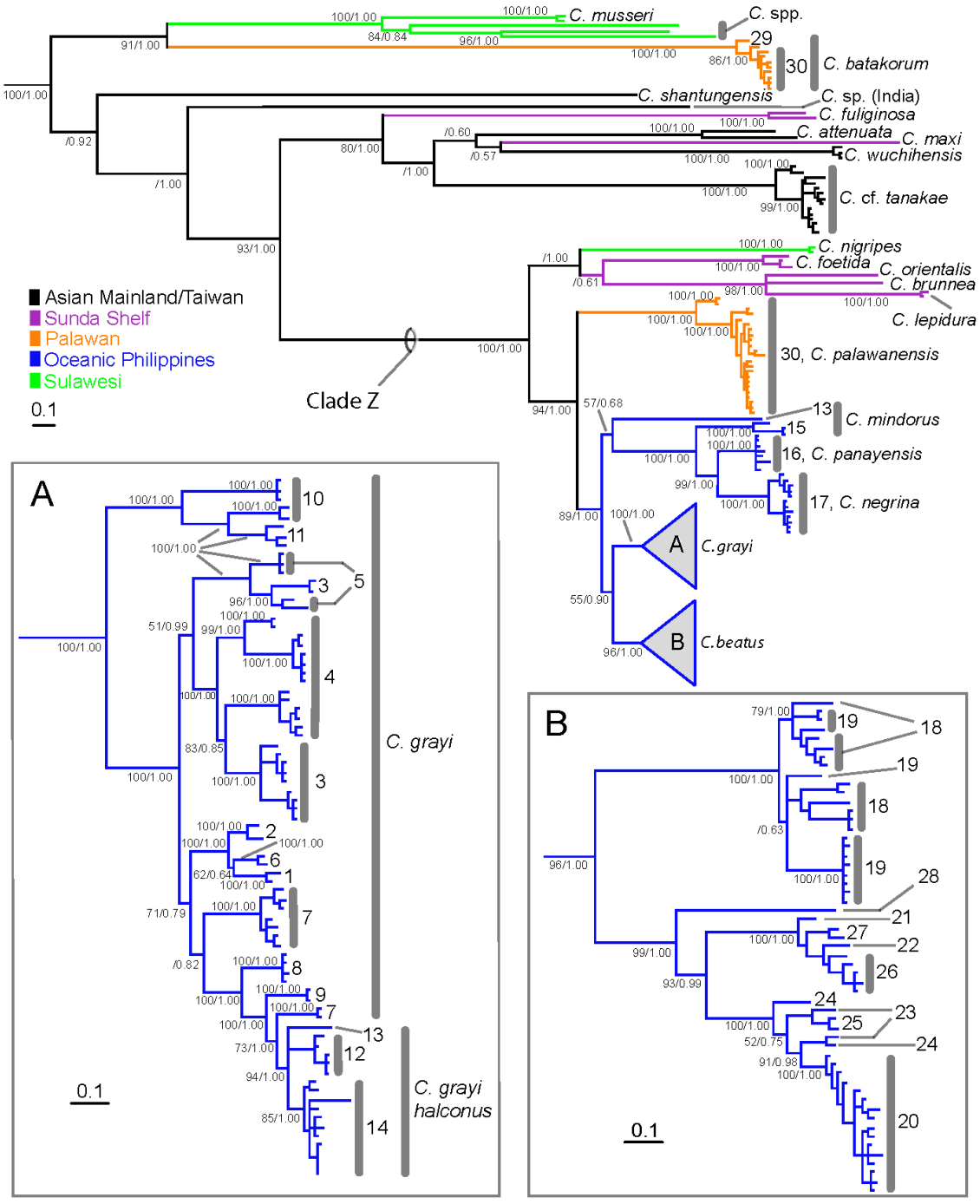
The concatenated data set consists of 4055 characters, 1143 of which are parsimony informative. Topological inferences among optimality criteria and individual loci vs. concatenated data sets are largely congruent, though some differences exist. Most discrepancies are in areas of the tree that receive low support and/or have short internal branches. The partitioned Bayesian analysis of the concatenated matrix yields a largely resolved topology with most nodes receiving strong support (Fig. 1.4). The analysis restricted to nuclear loci was consistent with the concatenated topology, but relationships within the main Philippine clade (excluding *C. batakorum*) are unresolved (not shown). The ultrametric tree based on our likelihood analysis of CytB (Fig. 1.5) yields a similar topology to that from the partitioned Bayesian analysis. However, the relative positions of the three clades that make up the oceanic Philippine group are shuffled, the position of

C. palawanensis has changed, and the clade that includes *C. foetida*, *C. nigripes*, and others is not inferred. All of these relationships received low support in the likelihood analysis restricted to CytB. In the Bayesian analysis of CytB (not shown), these relationships are inferred as in the combined analysis (Fig. 1.4).

Our topological inferences show three well-supported clades that include a basal group from Sulawesi and Palawan, a clade with a mixture of mainland Indochinese and Sunda Shelf taxa, and a clade that includes species from the Philippines, Sulawesi, and the Sunda Shelf (Clade Z; Fig. 1.4). A few species reside on long branches rooted in the basal portions of the tree.

Our analyses repeatedly recover three mostly allopatric clades that are distributed across the northern (*C. grayi* complex), central (*C. mindorus* + *C. negrina* + *C. panayensis*), and southern portions of the oceanic Philippines (*C. beatus* complex). The geographic distribution of these clades is congruent with earlier biogeographical delineations (e.g., Dickerson 1928; Heaney 1986). These clades are usually arranged with *C. beatus* and *C. grayi* sister to each other, with the central clade sister to the two, though support values for these relationships are always low and internode branches short.

Figure 1.4. Bayesian estimate of phylogenetic relationships among species and populations of Southeast Asian shrews (genus *Crocidura*) as inferred from a partitioned analysis of two mitochondrial and three nuclear genes. Numbers at the nodes indicate bootstrap values from a maximum parsimony analysis, followed by Bayesian posterior probabilities. The outgroup (*Suncus murinus*) and node support values from within populations were removed for clarity of presentation. Numbers at the terminal branches refer to Philippine collection localities denoted in Fig. 1.3.


C. musseri
C. spp.
C. batakorum
C. shantungensis
C. sp. (India)
C. fuliginosa
C. attenuata
C. maxi
C. wuchihensis
C. cf. tanakae
C. nigripes
C. foetida
C. orientalis
C. brunnea
C. lepidura
30, C. palawanensis
13
C. mindorus
15
16, C. panayensis
17, C. negrina
A
C.grayi
B
C.beatus
Clade Z
Asian Mainland/Taiwan
Sunda Shelf
Palawan
Oceanic Philippines
Sulawesi
0.1
A
C. grayi
C. grayi halconus
0.1
B
0.1

The Role of Inter-Island Colonization

Our evaluations of topological hypotheses provide several insights into the evolution of shrew diversity in Southeast Asia (Table 1.3). First, we soundly reject a single colonization event for the Philippines (including Palawan), but not for the oceanic portion of the archipelago (excluding Palawan). Second, the biogeographical position of Palawan in our phylogenetic analyses is not that of a simple extension of the Sunda Shelf. The clade that includes *C. batakorum* and *C. musseri* is shared between Palawan and Sulawesi, though this relationship could be altered with the addition of currently unavailable Sunda Shelf taxa. The other Palawan species (*C. palawanensis*) is part of a clade that includes all species from the oceanic Philippines, though it is sister to these. This relationship is well supported in the analyses of the concatenated matrix (Fig. 1.4) and by the Bayesian CytB analysis (not shown), but not recovered in the likelihood analysis of CytB (Fig. 1.5). *P*-values associated with the various *C. palawanensis*–Sunda Shelf sister relationship constraints are marginal (Table 1.3). However, with one relatively old Palawan species (*C. batakorum*) grouping with Sulawesi and one relatively young species (*C. palawanensis*) grouping with the oceanic Philippines, the characterization of the island group as an extension of Borneo is an over-simplification, a conclusion also reached by Brown and Diesmos (2009).

Neither of the syntopic Philippine species pairs (Palawan Island: *batakorum* and *palawanensis*; Mindoro Island: *grayi halconus* and *mindorus*) shows a sister relationship in any of our analyses and these hypotheses are rejected by our statistical tests (Table 1.3). It therefore appears that all speciation among currently named Philippine taxa has resulted from over-water colonization followed by divergence in allopatry. However, we

note that some species, especially *C. beatus*, are genetically variable and represent several independently evolving lineages. It is evident (Fig. 1.4B) that extensive within Pleistocene island diversification has occurred, but current taxonomy does not reflect this variation.

In contrast to the allopatric distribution of Philippine shrew diversity, Sulawesi supports an exceptionally high level of sympatric diversity; Ruedi (1995) reported capturing five species in a small area near the center of the island. Our study is consistent with the conclusion of Ruedi et al. (1998) that shrews colonized Sulawesi at least twice. Two distantly related lineages occur on the island. One is represented by a single species, *C. nigripes*. The other clade consists of a monophyletic assemblage of eight species, three of which are undescribed (Fig. 1.5). This is a remarkable level of shrew diversity, especially considering that the nine species were sampled from only two of seven areas of endemism identified by Evans et al. (2003b). Given this result, within-island speciation, and perhaps sympatric speciation, may have played a prominent role in the diversification of Sulawesian shrews.

Table 1.3. Results of Bayesian and Approximately Unbiased (AU) evaluation of topological hypotheses. Posterior probabilities (PP) and *p*-values are presented for the complete concatenated (Concat) and cytochrome-b matrices (CytB). Evaluation of the last hypothesis (Palawan part of Sunda Shelf) involved multiple independent constraints on the relationships of *C. batakorum* and *C. palawanensis*; only the highest *p*-value among six distinct constraints is presented. *P*-values significant at $\alpha \leq 0.05$ are denoted by bold text.

Hypothesis	**Constraint**	**PP Concat/CytB**	**AU Concat/CytB**
Single colonization of Philippines	Monophyletic Philippine clade	0/0	**<0.001/<0.001**
Single colonization of oceanic Philippines	Monophyletic oceanic Philippine clade	1/0.71	0.971/0.500
Single colonization of Sulawesi	Monophyletic Sulawesian clade	0/0	**<0.001/<0.001**
Within-island speciation on Mindoro	*halconus* & *mindorus* sister taxa	0/0	**<0.001/0.001**
Within-island speciation on Palawan	*batakorum* & *palawanensis* sister species	0/0	**<0.001/0.037**
Palawan part of Sunda Shelf	*batakorum* or *palawanensis* sister to any species from the Sunda Shelf	0/0	**0.037**/0.077

Temporal Patterns of Diversification

Log likelihood scores with the molecular clock enforced and not enforced were -13,953 and -13,827, respectively. The LRT gave a non-significant result (χ^2_{252}, $P = 0.49$) and we proceeded to use a standard molecular clock (Fig. 1.5). The two substitution rates (0.00562 and 0.01385/site/my) used to estimate divergence dates provide a wide range of possible ages, but both indicate that our assumptions regarding the arrival of shrews in Southeast Asia are probably valid. The lineage-through-time plots (LTT) of the entire data set and Clade Z are each suggestive of either a constant rate of diversification or a subtle decline in rates through time (Fig. 1.6). For both LTTs, rate-variable models received lower AIC scores (i.e., better fit; Table 1.4) than the best rate-constant model (pure birth). However, ΔAIC scores were not significant in either case (All taxa, ΔAIC = 3.1, $P = 0.14$; Clade Z, ΔAIC = 3.8, $P = 0.07$). Power analyses indicate that we would have a moderate probability of rejecting CRD if rates declined 2-fold and a high probability of rejecting the null under a 4-fold decline in rates (Fig. 1.7). Statistical power for detecting temporal increases in diversification rates was weaker, but a visual inspection of the LTTs indicates that temporal increases (PSL) are unlikely to represent a viable explanation of the data. We interpret these results as evidence that there is not a strong signal of diversification under the MPV or DDD hypotheses.

Table 1.4. Rate-constant and rate-variable models of diversification fit to the ultrametric phylogeny of shrews (Fig. 5). Model names as in LASER 2.0 (Rabosky 2006a). AIC scores are given for each of the empirical LTTs. AIC scores from the rate-constant and rate-variable models providing the best fit are noted with bold text, as are values for ΔAIC and *P*-values.

Model Name	**Rate Category**	**Free Parameters**	**Model Type**	**AIC All taxa**	**AIC Clade Z**
pureBirth	Constant	1	Yule	**-446.4**	**-196.4**
bd	Constant	2	Birth-death	-444.4	-194.4
yule2rate	Variable	3	Yule	**-449.5**	-198.6
rvbd	Variable	4	Birth-death	-447.5	-196.6
DDL	Variable	2	Density-dependent logistic	-448.0	**-200.2**
DDX	Variable	2	Density-dependent exponential	-446.7	-198.0
			ΔAIC	**3.1**	**3.8**
			P-value	**0.14**	**0.07**

Discussion

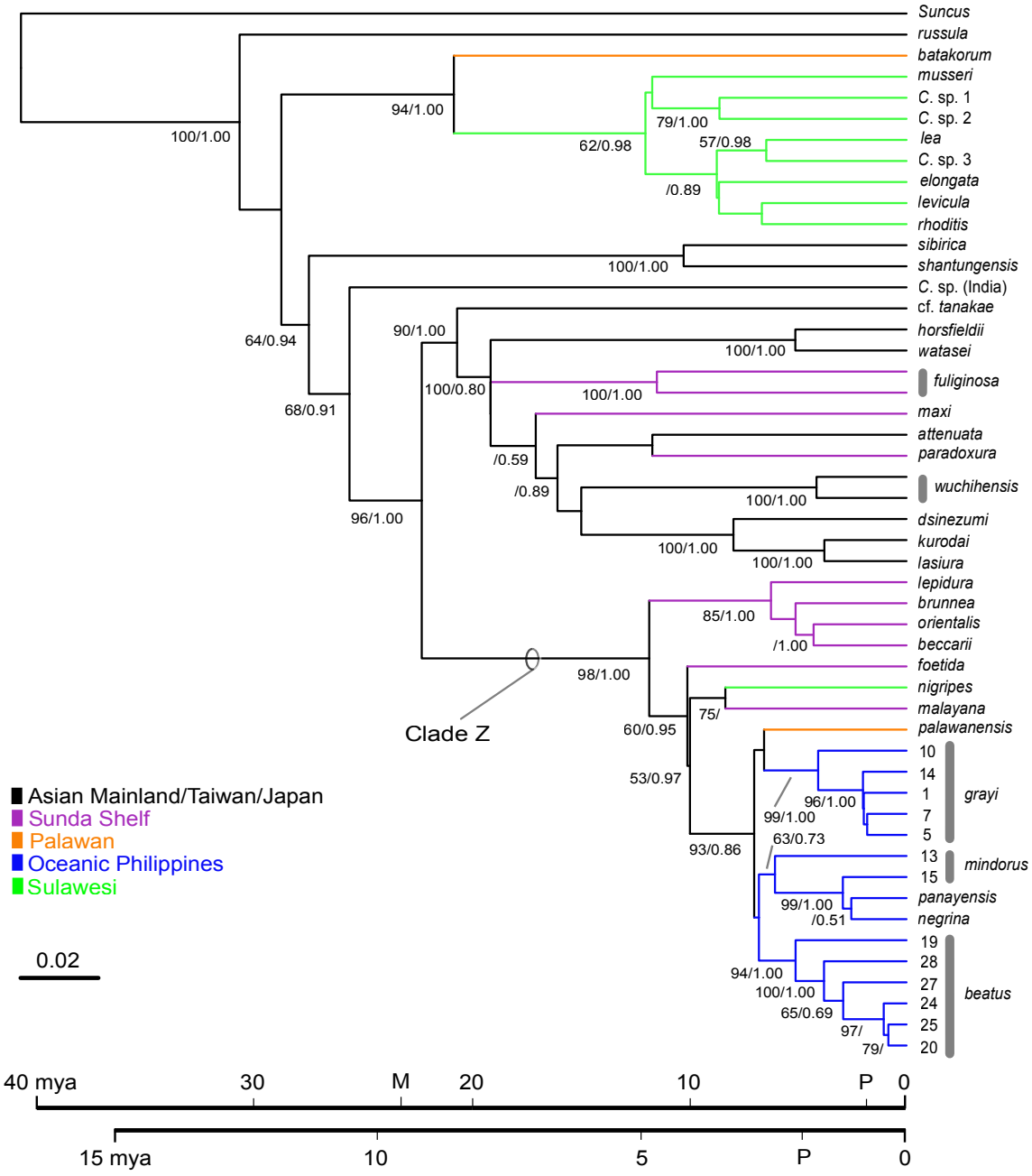
The Role of Inter-Island Colonization

Our topological inferences reveal a consistent pattern indicative of multiple invasions of most biogeographic regions. The Sunda Shelf holds multiple independent lineages of shrews. Our analyses using multiple loci and greater taxon sampling further support Ruedi et al.'s (1998) hypothesis that shrews colonized Sulawesi at least twice. The oceanic Philippines (i.e., excluding Palawan) apparently has been invaded only once, though extensive movements by shrews across water barriers within the Philippines are necessary to explain current distributions and phylogenetic relationships.

The Palawan group of islands, which has generally been considered a peripheral portion of the Sunda Shelf, shows some surprising biogeographical affinities. With respect to the phylogenetic relationships among shrews, Palawan clearly has ties to both Sulawesi and the oceanic Philippines, but not a close relationship to the Sunda Shelf. This is contra to the hypothesis of Heaney and Ruedi (1994) that *C. palawanensis* is a close relative of *C. fuliginosa* and not part of the oceanic Philippine radiation. The Palawan group is probably most appropriately viewed as having a complex of faunal affinities, with various lineages having close phylogenetic relationships to forms on Borneo, the oceanic Philippines, and Sulawesi (Brown and Diesmos 2009). The island chain may have played an important role as a colonization route into the oceanic Philippines for shrews and other taxa (Brown and Guttman 2002; Diamond and Gilpin 1983; Jones and Kennedy 2008).

All evidence from the Philippines indicates that currently recognized species are the result of over-water colonization events and subsequent divergence in allopatry.

Figure 1.5. An ultrametric, maximum likelihood phylogeny of Southeast Asian shrews inferred from cytochrome-b sequences and calibrated using two plausible substitution rates (see Materials and Methods). "P" and "M" on the time scales denote the beginning of the Pleistocene and Miocene, respectively. Redundant, within population sampling has been eliminated. Numbers at the nodes represent bootstrap support (when >50%) followed by Bayesian posterior probabilities. Numbers at the terminal branches refer to Philippine collection localities denoted in Fig. 1.3.


Suncus
russula
batakorum
musseri
C. sp. 1
C. sp. 2
lea
C. sp. 3
elongata
levicula
rhoditis
sibirica
shantungensis
C. sp. (India)
cf. tanakae
horsfieldii
watasei
fuliginosa
maxi
attenuata
paradoxura
wuchihensis
dsinezumi
kurodai
lasiura
lepidura
brunnea
orientalis
beccarii
foetida
nigripes
malayana
palawanensis
grayi
mindorus
panayensis
negrina
beatus
Clade Z
Asian Mainland/Taiwan/Japan
Sunda Shelf
Palawan
Oceanic Philippines
Sulawesi
0.02
40 mya
30
M
20
10
P
0
15 mya
10
5
P
0

However, if current taxonomy reflected phylogenetic diversity, then *C. beatus* and perhaps *C. grayi*, would be split into multiple taxonomic entities (species) distributed allopatrically across the Mindanao and Luzon faunal regions. We further note that sympatry among Philippine shrews is achieved only among older lineages and all sympatric species differ substantially in body size and perhaps ecologically (elevational segregation and tolerance of habitat disturbance), suggestive of the idea of a 'sympatry threshold' (e.g., Marshall et al. 2008).

In contrast, eight species from Sulawesi form a well-supported clade, indicating that within-island speciation, and perhaps sympatric speciation, may have played a significant role in the diversification process. However, modern Sulawesi is an aggregate of several once distinct islands (Hall 2002). A phylogenetic estimate calibrated with multiple sources of data (e.g., fossils, group-specific substitution rates, and geological events) might provide important information on the timing of the arrival of shrews relative to the timing of the coalescence of the once independent islands and to their rifting from earlier landmasses.

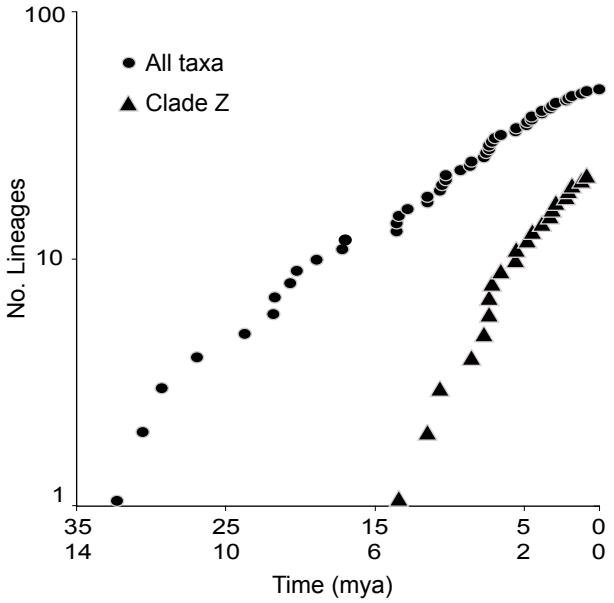


Figure 1.6. Lineage-through-time plots of Southeast Asian shrews derived from the phylogeny in Fig. 1.5. Noted are the diversification rates for the entire phylogeny (circles) and Clade Z (triangles). The time scales on the x-axis are generated from two plausible substitution rates (see Methods).

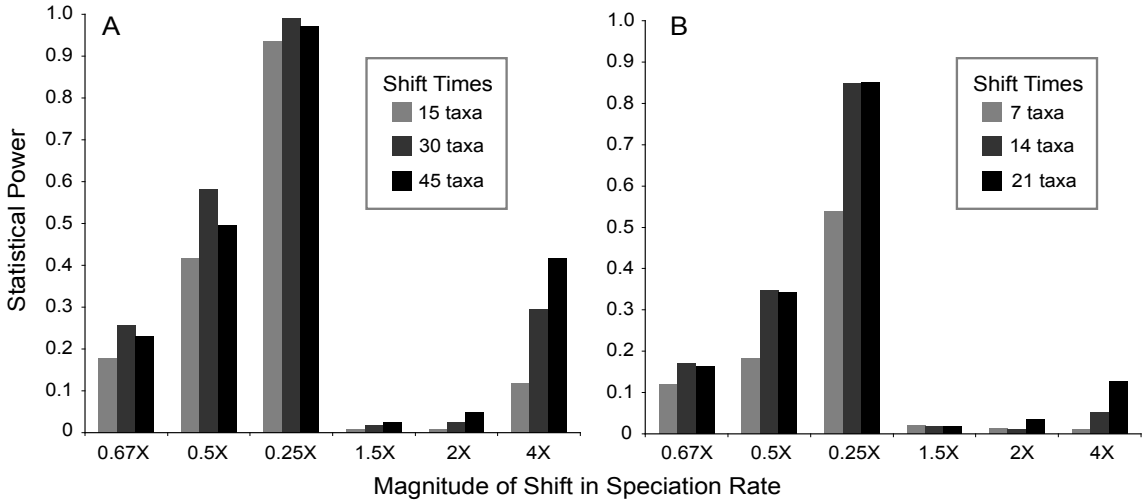


Figure 1.7. Probability of rejecting the null hypothesis of a constant rate of diversification (CRD) when birth rates decline and increase 1.5-, 2-, and 4-fold at three points in time in an expanding phylogeny. Statistical power is shown in simulated phylogenies with 49 taxa sampled from a clade of 61 species (**A)** and 23 taxa sampled from a clade of 29 species (**B**). Speciation rates shifted when the growing trees had 15, 30, and 45 terminals (**A**) and 7, 14, and 21 terminals (**B**).

Temporal Patterns of Diversification

Our birth-death analyses suggest that the net diversification rate has been relatively constant through time. Although models with declining rates provided the best fit, we are unable to reject the null, constant rate hypothesis. In contrast, most studies of diversification rates identify statistically significant temporal declines (Kozak et al. 2006; McPeek 2008; Phillimore and Price 2008; Price 2008).

The distribution of terrestrial habitats in Southeast Asia has been extremely dynamic through geological history (Bird et al. 2005; Defant et al. 1990; Hall 1998; Heaney 1985, 1986, 1991; Voris 2000) and two periods of time (Miocene–Pliocene and Pleistocene) are characterized by extensive changes in the distribution of land. The earlier period was a time of intensive volcanic uplift and numerous new islands were formed (Defant et al. 1990; Hall 1998; Ozawa et al. 2004). Afterward, during the Pleistocene, sea levels fluctuated extensively, repeatedly connecting and isolating many islands (Haq et al. 1987; Rohling et al. 1998; Voris 2000). Either process could have led to increased rates of diversification through the generation of new terrestrial habitats or intermittent connection of previously inaccessible lands. Our model-fitting analyses reject the notion that one of these processes had a strong effect on diversification rates. It is unlikely that biased or incomplete taxon sampling drives our conclusions because our separate tests of the entire phylogeny and Clade Z lead to the same interpretation. Incomplete, random taxon sampling makes the inference of declining rates of diversification more likely, whereas biased taxon sampling can affect results in a variety of ways (Nee 2001). We doubt that a sampling bias has caused our failure to reject the null hypothesis because we sampled 81% (25 of 31) of the species known from the area

occupied by Clade Z (Sunda Shelf, Sulawesi, and Philippines). Some species from the Sunda Shelf do not belong to Clade Z, thus we suspect that some of the six missing species also are not members of Clade Z. Therefore the total number of known species missing from this clade is likely fewer than six. For this small number of species to affect our results, there would need to be a very strong bias in their ages (e.g., all old lineages). Nevertheless, it remains a possibility that either there are many yet undiscovered species of *Crocidura* in Southeast Asia or that this clade has experienced a decline in speciation rates through time, but a high rate of extinction has eroded the signal (Rabosky and Lovette 2008).

We note that the LTTs (Fig. 1.6) suggest the net rate of diversification has been faster in Clade Z than in the entire phylogeny. Clade Z is entirely insular and this may reflect a difference in the rate of diversification between the islands and the continent. However, our limited sampling from the mainland prevents an explicit test of this hypothesis. Nevertheless, our inference of a relatively constant diversification rate through time in analyses of both the entire phylogeny and Clade Z, in the presence of apparent rate variation across geography, is intriguing.

If shrews have indeed diversified at a constant rate, two potential explanations are conceivable. First, the extreme heterogeneity (spatial and temporal) that characterizes large archipelagos may provide new opportunities for allopatric speciation over long periods of time. Second, an apparent constant rate of diversification could result from this group of shrews being an immature evolutionary radiation that has not existed long enough for the net diversification rate to plateau, as would be expected under a density-dependent model. These two hypotheses are not mutually exclusive, and the dynamic nature of large, old archipelagos may simply prolong the period of early, rapid speciation

commonly noted in continental clades. Kozak et al. (2006) suggested that niche conservatism plays a role in promoting the diversification of lineages, especially where extensive opportunities for diversification in allopatry exist. Species of *Crocidura* have undergone limited morphological and ecological diversification in most of Southeast Asia. We note that the region has an unusually high diversity of shrew-like rodents (especially on Luzon; e.g., *Rhynchomys*, *Archboldomys*) and this may constrain ecological diversification in shrews.

Taxonomic Hypotheses and Macroevolutionary Inferences

Macroevolutionary studies implicitly rely on a foundation of taxonomic hypotheses, which contain their own biases and limitations. Taxonomic decisions usually are based on exclusivity criteria, such as complete fixation of morphological differences and monophyly of gene trees (de Queiroz 1998). Fixation of characters and gene tree monophyly generally take long periods of time to form after cessation of gene flow (Knowles and Carstens 2007), indicating that we probably are unable to recognize the most recently formed species. Studies of the temporal pattern of diversification would therefore be expected to show a decline in diversification rates near the present because of their reliance on a taxonomy incapable of recognizing young species.

In this study, we use information from taxonomy, supplemented with information on genetic diversity, and find that a model with a constant rate of diversification provides good fit to the data. In contrast, most such studies find a strong pattern of temporally declining rates of diversification (McPeek 2008; Phillimore and Price 2008). Clearly, more clades would show constant rates, lesser declines, or even increasing rates of diversification through time if phylogeographic diversity were commonly considered in

concert with taxonomic information. It should be recognized that the limitations of taxonomic hypotheses (i.e., our inability to recognize young species), combined with the nature of stochastic birth-death processes (i.e., lineages that experience rapid, early diversification tend to be extant, diverse, and thus subject to phylogenetic estimation) may provide a viable explanation when temporally declining rates of diversification are inferred.

Conclusions

Southeast Asian shrews have diversified primarily through a process of repeated colonization of oceanic islands followed by divergence in allopatry, though the possibility remains that shrews speciated in sympatry on Sulawesi. The Sunda Shelf, Philippines (including Palawan), and Sulawesi all appear to have been colonized multiple times. Within the Philippines, shrews have colonized all major islands and substantial, within-island diversification has occurred on the large islands of Mindanao and Luzon (Fig. 1.4A, B). Closely related, unnamed lineages that inhabit these islands remain allopatric, but more distant relatives (species recognized by taxonomy) are sympatric or syntopic. In contrast, Sulawesi shrews may have diversified on a single paleoisland and of the nine species reported here, five are known to occur in sympatry (Ruedi 1995; Ruedi et al. 1998). Overall, diversification in this group has occurred in a manner consistent with a constant-rate, pure-birth process and with models that incorporate subtle declines in rates of diversification through time. There is not strong evidence that volcanic uplift during the Miocene and Pliocene (MPV hypothesis) resulted in an elevated diversification rate; the idea that Pleistocene sea-level fluctuations resulted in an increased diversification rate (PSL hypothesis) is probably not viable for this group. The

observation of a relatively constant rate of diversification is uncommon among studies that have explored the subject (McPeek 2008; Price 2008) and may reveal something unique about either the archipelago or the lineage under consideration. We suggest that (1) the dynamic history of Southeast Asia has generated a continuous supply of new opportunities for allopatric speciation, that (2) this group represents an immature radiation that has yet to fill geographical and ecological space, and/or (3) constant rates of diversification are in fact common, but rarely documented due to biases in taxonomic hypotheses and the nature of stochastic birth-death processes. Comparisons with other widespread Southeast Asian lineages should provide insights into which explanation(s) best accounts for the spectacular biodiversity of modern Southeast Asian archipelagos.

CHAPTER 2

The role of repeated sea-level fluctuations in the generation of shrew (Soricidae: *Crocidura*) diversity in the Philippine Archipelago

Geographic patterns of variation within lineages reveal basic features of the processes that generate and maintain biodiversity. These patterns, when considered in concert with well-substantiated phylogenetic hypotheses, illuminate evolutionary processes and have important implications for the conservation of biodiversity (Carstens et al. 2004; Evans et al. 2003b; Heaney et al. 2005). Observed patterns may lead to insights regarding the nature (e.g., allopatric vs. sympatric) and tempo of speciation, the temporal and spatial occurrence of barriers to gene flow, the nature of demographic parameters through time, and the appropriate partitioning of diversity into taxonomic units.

The extent of connections among modern islands during Pleistocene (and earlier) sea level low stands has long been recognized as an important factor in the evolution and assembly of biodiversity in the Philippines and on the Sunda and Sahul shelves (Delacour and Mayr 1946; Dickerson 1928; Heaney 1985; Inger 1954; Kloss 1929; Simpson 1977; Voris 2000). Deep-water channels generally separate distinctive biological communities, whereas neighboring islands currently separated by shallow water tend to share largely similar biotas (Brown and Diesmos 2002; Dickerson 1928; Esselstyn et al. 2004; Heaney 1986; Heaney et al. 1998). These shallow-water islands experienced repeated bouts of connection and isolation due to Pleistocene sea-level fluctuations, the magnitude of which ranged from 100 to 140 m below current sea levels (Rohling et al. 1998). During

periods of low sea level, five major islands existed in the Philippines; these are referred to as Pleistocene Aggregate Island Complexes (PAICs: Brown and Diesmos 2002).

The commonly observed pattern of faunal similarity among islands within PAICs and sharp differences between faunas on neighboring complexes (Dickerson 1928; Heaney 1986; Heaney et al. 1998) implies that gene flow within PAICs has been common, if intermittent. However, because the role of Pleistocene geography has long been recognized, there is a risk that taxonomic decisions could have been based in part on PAIC geography, and PAIC importance then inferred from taxonomy, thereby resulting in an over-emphasis of the importance of Pleistocene sea-level fluctuations. Thus, there is a need to evaluate the spatial distribution of genetic, morphological, and ecological diversity delimited by criteria independent of PAIC geography. Surprisingly few studies have attempted to do so (though see Brown and Guttman 2002; Evans et al. 2003a; Heaney et al. 2005; Roberts 2006a, b), leaving open the question of how pervasive the influence of PAIC geography might have been.

An ideal system for testing for the effects of intermittent land connections on the diversification process would be an organism that: (1) is present on all islands; (2) is short lived with a rapid rate of substitution (so that genetic signal will be detectable); (3) has a limited ability to cross sea channels; and (4) is commonly collected during biodiversity surveys. Shrews (Soricidae: *Crocidura*) fit this ideal in many respects. They are known from all major islands that have been surveyed for small, non-volant mammals in the Philippines (Esselstyn et al. 2009; Heaney and Ruedi 1994); they are short-lived, which may result in a rapid rate of molecular evolution; and they have a small body size and high metabolic rate, presumably making them relatively poor over-water colonizers.

Ruedi (1996) used allozyme data to explore diversity in shrews on the Sunda Shelf and the oceanic islands of the Philippines and Sulawesi. He found that isolation by distance failed to explain diversity throughout the region, but that distance explained a significant proportion of variation when the analysis was restricted to the Sunda Shelf. We interpret this to indicate that intermittent, shallow-water barriers isolating islands of the Sunda Shelf have been insignificant in the generation of diversity (relative to distance), whereas, deep-water channels isolating the Philippines and Sulawesi represent significant barriers to dispersal. In contrast, Gorog et al. (2004) considered movements of rodents across lowland areas of Borneo to have been rare during the Pleistocene, perhaps due to aridification and the limited distribution of forests (Bird et al. 2005; Heaney 1991), implying that land connections on their own, may not be sufficient to provide for dispersal.

Since the publication of Ruedi's (1996) work, the number of *Crocidura* specimens from the region has increased significantly and recent studies (Dubey et al. 2008; Esselstyn et al. 2009) provide phylogenetic context, allowing a test of these patterns within the Philippine archipelago. Nine species of *Crocidura* currently are recognized in the Philippines (Heaney and Ruedi 1994; Hutterer 2007). Esselstyn et al. (2009) include seven of these in phylogenetic inferences. Six Philippine species form a well-supported, widespread, monophyletic group (*beatus*, *grayi*, *mindorus*, *negrina*, *palawanensis*, and *panayensis*). Of the remaining species, two (*batakorum* and *attenuata*) probably represent separate invasions of the archipelago and one (*grandis*) has not been seen since the holotype was collected in 1906 (Esselstyn et al. 2009; Heaney and Ruedi 1994; Miller 1910).

If we assume that the history and geography of PAICs was the dominant factor in the evolution of Philippine biodiversity, several predictions may be derived, including: (1) populations on modern islands will be most closely related to adjacent island populations within the PAIC; (2) individual PAICs will hold monophyletic lineages (though gene trees will not always reflect this); (3) degree of genetic divergence between populations on different PAICs will be greater than those between populations residing on the same PAIC; and (4) populations separated by shallow water will have divergence dates associated with the end of the last glacial maximum (LGM).

In this study, we use time-calibrated phylogenetic estimates, analyses of molecular variance (AMOVAs), Mantel tests, and phylogeographic summary statistics to test for an effect of sea-level fluctuations on the generation of genetic diversity within the widespread Philippine clade. In particular, we explore patterns of genetic diversity to address the following questions: (1) Is genetic diversity in shrews partitioned primarily by PAICs and secondarily by islands within these complexes? (2) Do any of the divergences separating populations on neighboring islands within PAICs date to the end of the LGM, when rising sea levels last separated these islands? (3) Do other factors (e.g., isolation by distance and island area) contribute to genetic diversity?

To determine the generality of our conclusions, we make comparisons to a published study of genetic diversity in a small fruit bat endemic to the Philippines, (*Haplonycteris fischeri*; Roberts 2006b). Given the major differences in natural history between these two lineages (e.g., dispersal capacity, life span, and reproductive rates), any similarities in their patterns of genetic diversity might indicate pervasive causes. However, we note that *Crocidura* and *Haplonycteris* are not different in all aspects. For instance, both are probably most abundant in mid-elevation forests and moderately

tolerant of habitat disturbances (Heaney et al. 1998; Roberts 2006b). Although we compare patterns within a single named species (*H. fischeri*) to a clade of six named species (*Crocidura*), levels of genetic divergence among island populations within these two groups are similar, suggesting that either different taxonomic standards have been applied to these groups, or the extent of morphological diversification has been greater in *Crocidura*.

Our results show that PAICs explain some genetic variation and hence evolutionary history. However, the proportions explained in *Crocidura* are much less than those noted by Roberts (2006b) in *H. fischeri*. Phylogenetic topology in *Crocidura* fits the PAIC model well, but some divergence dates almost certainly predate the LGM. We further note that the inference of the time of speciation events is dependent on the calibration point used, and choosing among calibration points that produce wildly disparate estimates is difficult. Given our results, it is apparent that Pleistocene sea-level fluctuations are an important factor influencing patterns of variation, but they operated in a context where island area, isolation, and topographic relief, along with features of the organisms themselves, and perhaps other variables, must be taken into account.

Methods

We combined mtDNA sequence data from several sources (Bannikova et al. 2006; Brandli et al. 2005; Dubey et al. 2007a, b, 2008; Esselstyn et al. 2009; Ohdachi et al. 2006; Ohdachi et al. 2004; Ruedi et al. 1998) to explore patterns of genetic diversity of shrews within the Philippine Archipelago (see Appendix for details). We follow the taxonomy of Heaney and Ruedi (1994) and Hutterer (2007). Populations were sampled on all major Philippine islands and several small islands; we sampled multiple

populations from the large islands of Luzon, Mindanao, and Mindoro (Fig. 2.1). Based on the results described in Esselstyn et al. (2009), we assign newly discovered populations from Calayan and Samar islands to *C. grayi* and *C. beatus*, respectively.

We simultaneously estimated phylogenetic relationships and divergence dates using sequences of the mitochondrial gene, Cytochrome B (CytB). We included all taxa from the species-level alignment of Esselstyn et al. (2009) and added one terminal for all additional species found in Dubey et al.'s (2008) Old World + Asian *Crocidura* clade, including *Diplomesodon*. We included *Suncus murinus* in the analyses to serve as outgroup. These analyses were conducted in BEAST 1.4.8 (Drummond and Rambaut 2007) using the Yule speciation model and relaxed uncorrelated lognormal clock with sequences partitioned into 1^{st} + 2^{nd} and 3^{rd} codon positions. The GTR + I + Γ model of sequence evolution was chosen using the AIC criterion in MODELTEST (Posada and Crandall 1998). Parameter estimates were unlinked between the two partitions. Analyses were initiated with an UPGMA starting tree and run for 2×10^7 generations with trees and parameters sampled every 2000 generations. We examined trace files and effective sample sizes of parameters drawn from MCMC chains in Tracer (Rambaut and Drummond 2007) and compared posterior probabilities of splits between independent runs in AWTY (Nylander et al. 2008) to check for evidence of stationarity and convergence. The first 50% of each run was discarded as burn-in. We applied five calibration strategies to these analyses. All initial calibration strategies relied on normally distributed prior probabilities on the ages of particular nodes in the tree or on the substitution rate. We calibrated analyses with the oldest known fossil *Crocidura* (5.03 My ago: Butler 1998), secondary calibrations from a recent higher-level phylogenetic analysis (5.75 My ago origin of Old World + Asian *Crocidura*; 4.39 My

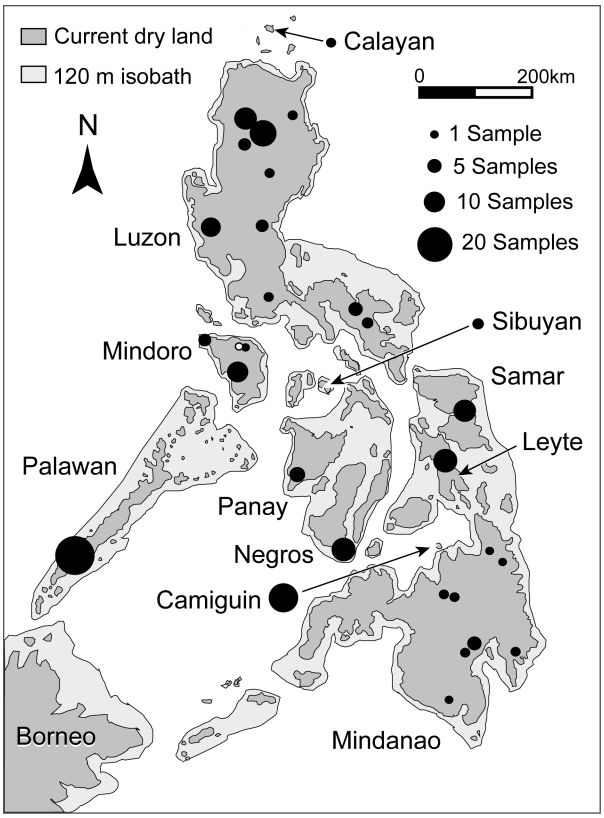


Figure 2.1. Distribution of shrew samples from the Philippines. Sample size is indicated by the diameter of the circle. On the island of Mindoro, we sampled two species; *C. mindorus* is noted with a white circle and *C. grayi halconus* with black circles. The modern distribution of land is shown in medium grey with the shorelines during Pleistocene sea-level low stands represented by the 120 m isobath indicated by light grey (after Heaney 1985).

ago origin of a clade found in the Philippines and Sunda Shelf: Fig. 1 of Dubey et al. 2008), and three geological calibrations from the Philippines. The geological calibration points were the uplift of Camiguin Island, occurring primarily around 0.35 My ago (Heaney and Tabaranza 2006; Sajona et al. 1997), the uplift of the Samar + Leyte region ca. 3 My ago (Sajona et al. 1997), and the collision of the Bicol Peninsula with Luzon Island ca. 3 My ago (Hall 2002). Each of these ages was used as a calibration point for the most recent common ancestor of the shrew population residing on that block and its sister group. We used 0.5 My as an arbitrarily determined standard deviation for the fossil, secondary, and geological calibrations. We ran additional analyses placing a prior probability on the substitution rate. These relied on the average mammalian rates for synonymous and nonsynonymous substitutions in CytB, determined from Figure 2 of Pesole et al. (1999). We calculated the proportions of each type of substitution in the *Crocidura* CytB matrix using DnaSP (Rozas and Rozas 1999) and used these values to calculate a weighted average of the mammalian rates. We then used a normally distributed prior on the per-site substitution rate with a mean of 0.009695 My^{-1} and standard deviation of 0.002 My^{-1}. The standard deviation was arbitrarily determined, but our intent was to encompass the range of variation known from mammals (Gissi et al. 2000; Pesole et al. 1999). Two independent runs were completed for each calibration strategy and the final 5000 trees from each run combined to calculate maximum clade credibility trees, posterior probabilities, median node ages, and 95% highest posterior densities of node ages.

A combined analysis that used all of the above calibration strategies was then employed. We changed the oldest fossil *Crocidura* calibration to a uniform prior with a range of 5–50 My so that it would function as a minimum calibration point. All other

priors were used as above. Four runs with these priors were undertaken for 2.5 x 10^7 generations. Because of strong conflict among the priors, we were forced to begin each run with a tree that resembled the 'correct' topology. We therefore started each run using the topology with the highest likelihood from the island-age calibrated runs. Again, the first 50% of samples were discarded and the maximum clade credibility tree was computed.

We then posed the question: does the phylogenetic association between island populations conform to the PAIC model more than would be expected by chance? We considered topologies to conform to the PAIC model if all island populations were most closely related to other island populations within their respective PAIC. We excluded the population of *C. beatus* from Camiguin Island because the phylogenetic relations of populations on small, oceanic islands are not informative with regard to the importance of PAICs in structuring genetic diversity. We considered the proportion of possible unrooted trees with 10 terminals (10 modern islands sampled from the Philippines, excluding Camiguin) in which taxa 1, 2, and 3 (Samar, Leyte, and Mindanao) form a monophyletic group, as do taxa 4 and 5 (Negros and Panay). We counted the number of possible trees consistent with this constraint in PAUP 4.0b10 (Swofford 1999) and divided this by the total number of possible trees with 10 terminals.

We then used a concatenated matrix of 1019 nucleotides of CytB and 1018 nucleotides of ND2 from 173 specimens (Fig. 2.1), representing six currently recognized species from the Philippines (Appendix). This matrix is complete with no missing characters. We computed several indices of genetic diversity, including the number of haplotypes, nucleotide diversity (π), and uncorrected genetic distance (p), using Arlequin 3.1 (Excoffier et al. 2005).

To determine whether divergences between island populations within PAICs could have originated at the end of the LGM, we calculated the rate of molecular evolution necessary to generate the observed, uncorrected genetic divergence between these populations. This was undertaken for the divergence between the Samar/Leyte clade and the Mindanao populations of *C. beatus*, and between *C. negrina* and *C. panayensis*. We used 10,000 (10K) years ago as the approximate time when rising waters would have separated these islands (Siddall et al. 2003; Voris 2000).

Three-way analyses of molecular variance (AMOVAs) were implemented to evaluate the role of Pleistocene sea-level fluctuations in the generation of genetic diversity. AMOVAs were completed with 1000 permutations in Arlequin 3.1 to explore genetic diversity across the entire archipelago with sequences partitioned by current taxonomy, PAICs, and modern islands. We also subjected the *C. grayi* and *C. beatus* complexes to independent AMOVAs, with data partitioned by modern islands and sample sites. Because we observed a large difference between *Crocidura* and *Haplonycteris* in the contribution of PAICs to genetic variation, we wanted to know how much of this difference might be due to disparities in the geographical distribution of samples available for these two lineages. We therefore repeated 10 iterations of the AMOVA of PAICs/modern islands/populations on reduced data sets. These jackknifed data sets were generated by removing randomly selected haplotypes (36–40% of all haplotypes were removed per iteration) and revealed the potential effects of variation in geographic sampling. Because Luzon Island was densely sampled and no other islands within the Luzon PAIC were represented, we analyzed the jackknifed data sets with all Luzon samples excluded.

We then tested for an association between geographic and genetic distances using Mantel tests (Mantel 1967). Latitude and longitude were taken from museum catalogs, specimen tags, or the field notes of collectors. Specimens sampled within 5 km of each other were considered members of the same population. A matrix of geographic straight-line distances among populations (including over-water distance when relevant) was generated using ArcGIS tools (Beyer 2004). Mean among population genetic distances were generated in Arlequin 3.1. Mantel tests were completed in the R package, APE (Paradis et al. 2004; R Development Core Team 2009), and relied on 5000 permutations to evaluate significance. We applied these methods to (1) all populations of *C. grayi*, (2) *C. grayi* from Luzon Island only, (3) all *C. beatus*, and (4) *C. beatus* from Mindanao Island only.

To test for an effect of island area on diversity, we plotted the per-population nucleotide diversity against the logarithm of island area and fit a least-squares regression to these data. Island areas were garnered from Heaney et al. (2002) and Allen et al. (2006).

Results

Phylogenetic analyses resulted in widely varying age estimates for clades of interest. Fossil (Fig. 2.2) and secondary calibrations (not shown) produced similar results, as did geological (not shown) and substitution rate (Fig. 2.3) priors. However, the former two produced much younger inferences than the latter two. The analysis combining all calibration points resulted in intermediate age estimates (Fig. 2.4).

The probability of a phylogeny with randomly determined relationships showing Samar + Leyte + Mindanao and Negros + Panay relationships is 0.001. Thus, the

topological relationships show a greater concordance to PAIC geography than would be expected by chance alone (Fig. 2.5).

One hundred and six haplotypes were identified among 173 mitochondrial sequences for an overall haplotype diversity of 0.6127. Of the 106 haplotypes, 72 were represented by a single individual, 14 by 2 individuals, 18 by 3–4 individuals, and 2 by 7 individuals (Table 2.1).

If the levels of genetic divergence among islands within PAICs (Fig. 2.6) are the result of 10K years of isolation, then rates of substitution necessary to generate the observed divergences between populations on Negros and Panay islands and between Samar/Letye and Mindanao islands would be 1.39 and 2.82 site^{-1} My^{-1}, respectively. These are two orders of magnitude faster than those typically reported for mammals (Fumagalli et al. 1999; Gissi et al. 2000). Thus, we suggest that a divergence as recent as the end of the last glacial maximum (LGM) is highly unlikely for these populations. If these divergences occurred after the Pliocene–Pleistocene boundary, substitution rates would be approximately ≥ 0.0077 and ≥ 0.0157 site^{-1} My^{-1}, calculations more consistent with what is thought of as typical mammalian rates (Pesole et al. 1999). Thus, it is plausible that these divergences occurred during the early–middle Pleistocene (Figs. 2.2–2.4), but extremely unlikely that they occurred after the LGM. However, we note that the populations on Samar and Leyte are genetically indistinguishable and gene flow may have occurred between these two islands during the LGM.

The proportions of genetic variation accounted for by taxonomy, PAICs, and modern islands are similar (48–54%), as are the among-population and within-population comparisons across these three partitioning strategies, at 35–42% and 10–11% (Fig. 2.7),

Table 2.1. Summary of mtDNA sequence diversity in *Crocidura* on Philippine islands.

Species	**Island**	**Area (km^2)**	**Populations Sampled**	**Haplotype Diversity**	**Nucleotide Diversity**
C. beatus	Camiguin	265	1	0.5625	0.0020
	Leyte	7213	1	0.4167	0.0074
	Samar	13,429	1	0.7273	0.0097
	Mindanao	96,467	8	0.875	0.0227
C. grayi	Calayan	196	1	1.0	0.0015
	Mindoro	9,735	3	0.6667	0.0054
	Luzon	107,170	10	0.625	0.0279
C. mindorus	Sibuyan	449	1	0.6667	0.0065
C. negrina	Negros	13,670	1	0.4167	0.0051
C. palawanensis	Palawan	11,875	1	0.4348	0.0046
C. panayensis	Panay	12,300	1	0.8333	0.0020

Figure 2.2. Maximum clade credibility tree from a phylogenetic analysis of *Crocidura*. This tree was calibrated with the oldest known fossil *Crocidura*. Numbers at nodes represent median age estimates in millions of years and medium grey bars represent the 95% highest posterior density of age estimates. Black diamonds indicate node support of ≥90% posterior probability. The vertical, light-grey bar represents the Pleistocene Epoch. Terminals are labeled with taxonomic names, followed by abbreviated localities (CH = China, GR = Greece, GU = Guinea, HU = Hungary, IC = Ivory Coast, ID = Indonesia, IN = India, IR = Iran, JP = Japan, LI = Libya, MA = Malta, MY = Malaysia, PH = Philippines, PM = Peninsular Malaysia, RU = Russia, RY = Ryukyu Islands, TH = Thailand, TW = Taiwan, VT = Vietnam).

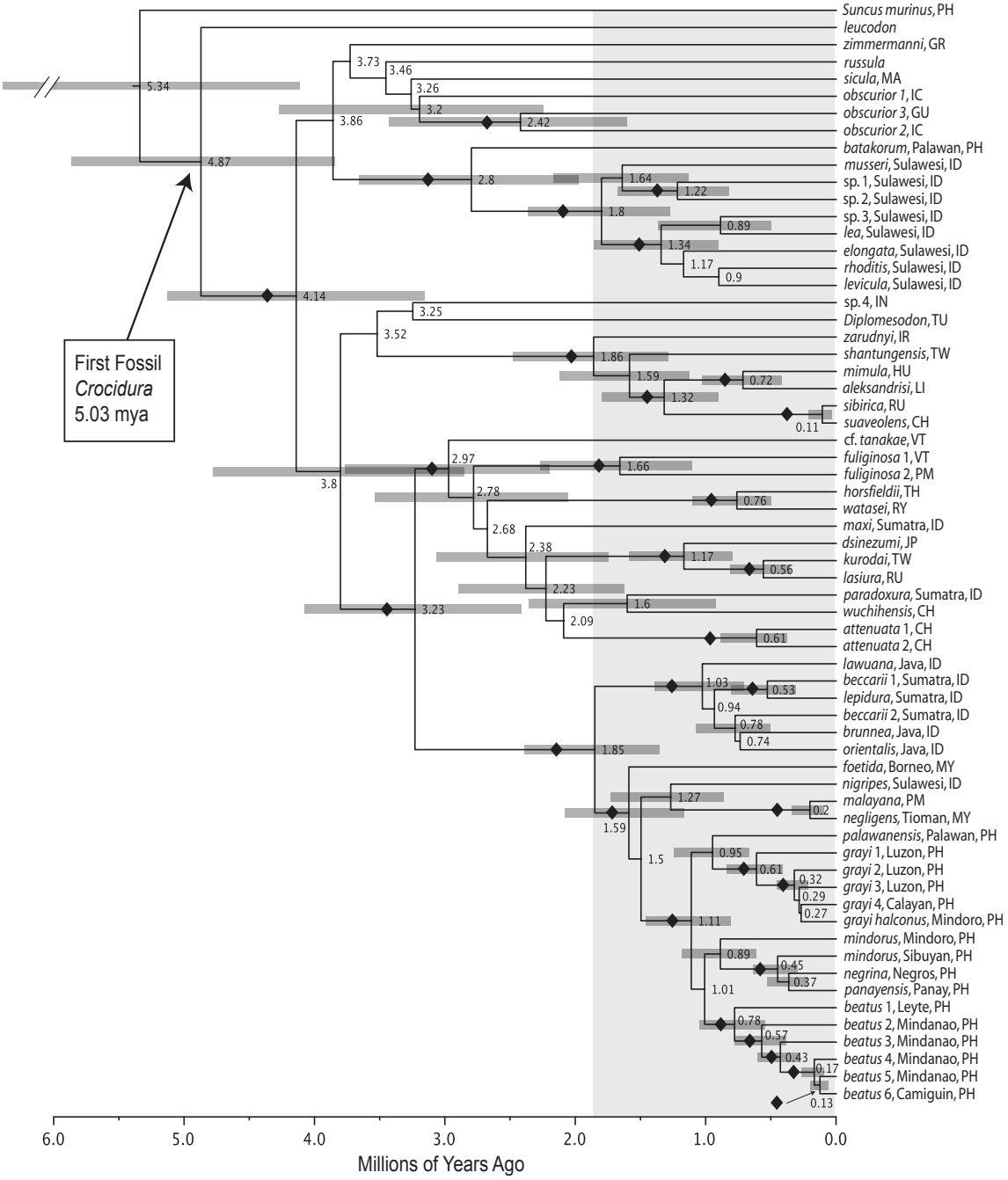

First Fossil
Crocidura
5.03 mya
Suncus murinus, PH
leucodon
zimmermanni, GR
russula
sicula, MA
obscurior 1, IC
obscurior 3, GU
obscurior 2, IC
batakorum, Palawan, PH
musseri, Sulawesi, ID
sp. 1, Sulawesi, ID
sp. 2, Sulawesi, ID
sp. 3, Sulawesi, ID
lea, Sulawesi, ID
elongata, Sulawesi, ID
rhoditis, Sulawesi, ID
levicula, Sulawesi, ID
sp. 4, IN
Diplomesodon, TU
zarudnyi, IR
shantungensis, TW
mimula, HU
aleksandrisi, LI
sibirica, RU
suaveolens, CH
cf. tanakae, VT
fuliginosa 1, VT
fuliginosa 2, PM
horsfieldii, TH
watasei, RY
maxi, Sumatra, ID
dsinezumi, JP
kurodai, TW
lasiura, RU
paradoxura, Sumatra, ID
wuchihensis, CH
attenuata 1, CH
attenuata 2, CH
lawuana, Java, ID
beccarii 1, Sumatra, ID
lepidura, Sumatra, ID
beccarii 2, Sumatra, ID
brunnea, Java, ID
orientalis, Java, ID
foetida, Borneo, MY
nigripes, Sulawesi, ID
malayana, PM
negligens, Tioman, MY
palawanensis, Palawan, PH
grayi 1, Luzon, PH
grayi 2, Luzon, PH
grayi 3, Luzon, PH
grayi 4, Calayan, PH
grayi halconus, Mindoro, PH
mindorus, Mindoro, PH
mindorus, Sibuyan, PH
negrina, Negros, PH
panayensis, Panay, PH
beatus 1, Leyte, PH
beatus 2, Mindanao, PH
beatus 3, Mindanao, PH
beatus 4, Mindanao, PH
beatus 5, Mindanao, PH
beatus 6, Camiguin, PH
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Millions of Years Ago

Figure 2.3. Maximum clade credibility tree from a phylogenetic analysis of *Crocidura*. This tree was calibrated with a normal prior on the substitution rate (mean = 0.009695, SD = 0.002 site^{-1} My^{-1}). Numbers at nodes represent median age estimates in millions of years and medium grey bars represent the 95% highest posterior density of age estimates. Black diamonds indicate node support of ≥90% posterior probability. The vertical, light-grey bar represents the Pleistocene Epoch. Terminals are labeled with taxonomic names, followed by abbreviated localities (CH = China, GR = Greece, GU = Guinea, HU = Hungary, IC = Ivory Coast, ID = Indonesia, IN = India, IR = Iran, JP = Japan, LI = Libya, MA = Malta, MY = Malaysia, PH = Philippines, PM = Peninsular Malaysia, RU = Russia, RY = Ryukyu Islands, TH = Thailand, TW = Taiwan, VT = Vietnam).

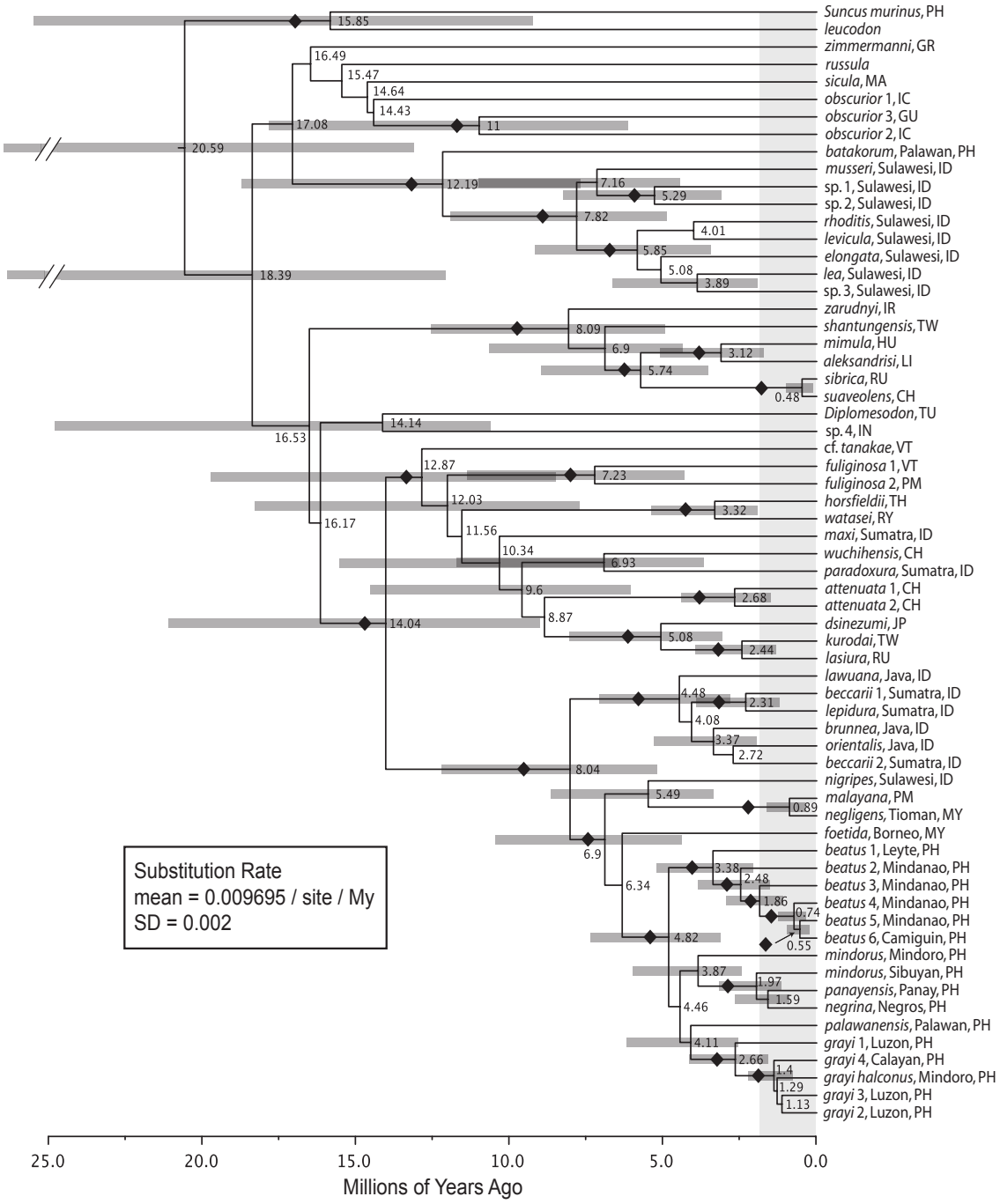
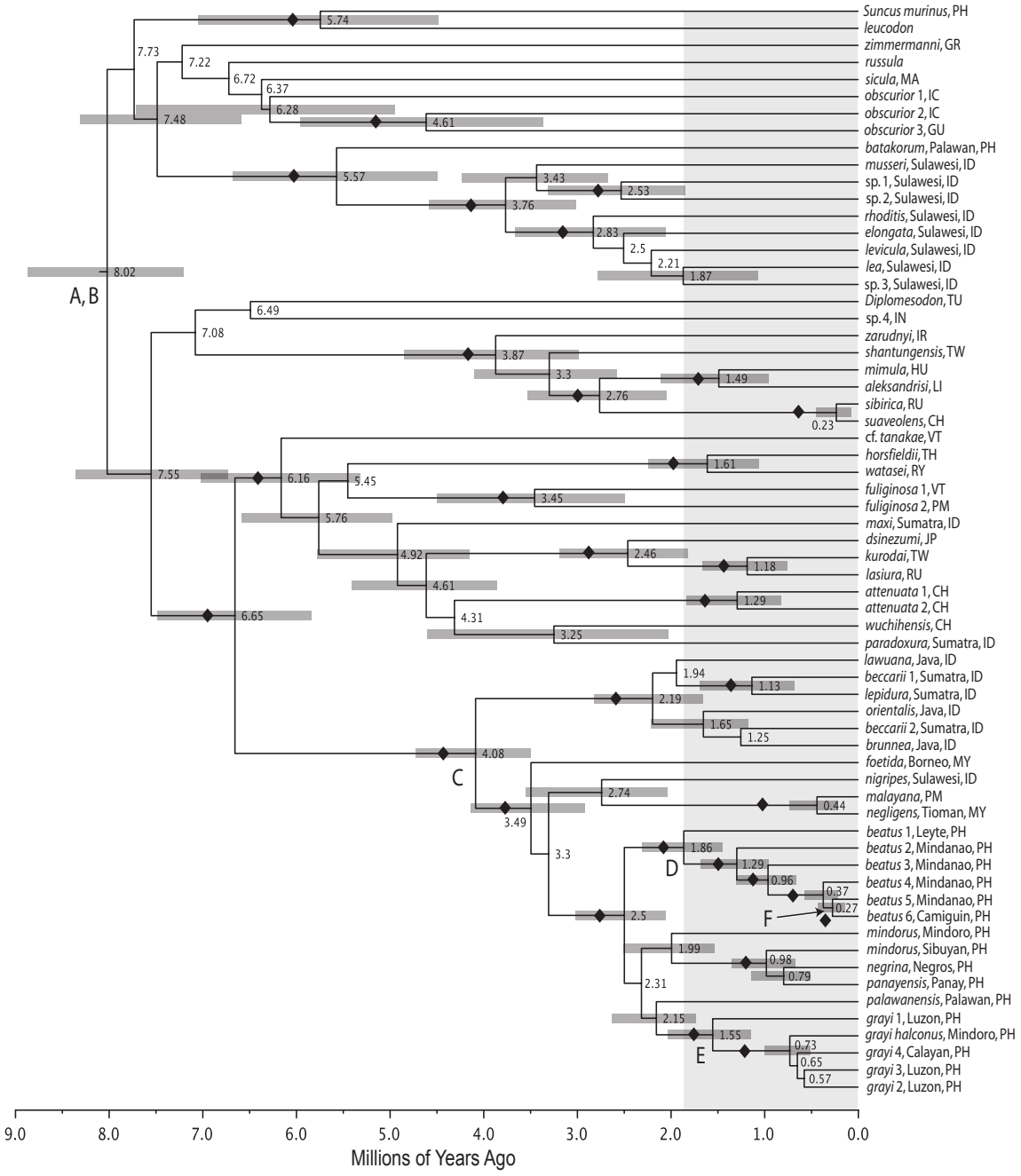

Suncus murinus, PH
leucodon
zimmermanni, GR
russula
sicula, MA
obscurior 1, IC
obscurior 3, GU
obscurior 2, IC
batakorum, Palawan, PH
musseri, Sulawesi, ID
sp. 1, Sulawesi, ID
sp. 2, Sulawesi, ID
rhoditis, Sulawesi, ID
levicula, Sulawesi, ID
elongata, Sulawesi, ID
lea, Sulawesi, ID
sp. 3, Sulawesi, ID
zarudnyi, IR
shantungensis, TW
mimula, HU
aleksandrisi, LI
sibrica, RU
suaveolens, CH
Diplomesodon, TU
sp. 4, IN
cf. tanakae, VT
fuliginosa 1, VT
fuliginosa 2, PM
horsfieldii, TH
watasei, RY
maxi, Sumatra, ID
wuchihensis, CH
paradoxura, Sumatra, ID
attenuata 1, CH
attenuata 2, CH
dsinezumi, JP
kurodai, TW
lasiura, RU
lawuana, Java, ID
beccarii 1, Sumatra, ID
lepidura, Sumatra, ID
brunnea, Java, ID
orientalis, Java, ID
beccarii 2, Sumatra, ID
nigripes, Sulawesi, ID
malayana, PM
negligens, Tioman, MY
foetida, Borneo, MY
beatus 1, Leyte, PH
beatus 2, Mindanao, PH
beatus 3, Mindanao, PH
beatus 4, Mindanao, PH
beatus 5, Mindanao, PH
beatus 6, Camiguin, PH
mindorus, Mindoro, PH
mindorus, Sibuyan, PH
panayensis, Panay, PH
negrina, Negros, PH
palawanensis, Palawan, PH
grayi 1, Luzon, PH
grayi 4, Calayan, PH
grayi halconus, Mindoro, PH
grayi 3, Luzon, PH
grayi 2, Luzon, PH
15.85
16.49
15.47
14.64
14.43
17.08
11
20.59
12.19
7.16
5.29
7.82
4.01
5.85
5.08
3.89
18.39
8.09
6.9
3.12
5.74
0.48
14.14
16.53
12.87
7.23
12.03
3.32
16.17
11.56
10.34
6.93
9.6
2.68
8.87
14.04
5.08
2.44
4.48
2.31
4.08
3.37
2.72
8.04
5.49
0.89
6.9
3.38
2.48
6.34
1.86
0.74
4.82
0.55
3.87
1.97
1.59
4.46
4.11
2.66
1.4
1.29
1.13
Substitution Rate
mean = 0.009695 / site / My
SD = 0.002
25.0
20.0
15.0
10.0
5.0
0.0
Millions of Years Ago

Figure 2.4. Maximum clade credibility tree from a phylogenetic analysis of *Crocidura*. This tree was calibrated with a combination of available strategies, indicated by letters at nodes: A = oldest fossil *Crocidura* (uniform distribution 5–50 My ago); B and C = secondary calibrations from Dubey et al. (2008: normal distribution with mean = 5.75 My ago, SD = 0.5 My and 4.39 My ago, SD = 0.5 My, respectively); D and E = geological calibrations from uplift of Leyte and collision of Bicol Peninsula with Luzon (each at mean = 3 My ago, SD = 0.5 My); F = uplift of Camiguin (0.35 My ago, SD = 0.5 My); and finally with a normal prior on the substitution rate (mean = 0.009695 site^{-1} My^{-1}, SD =0.002). Numbers at nodes represent median age estimates in millions of years and medium grey bars represent the 95% highest posterior density of age estimates. Black diamonds indicate node support of ≥90% posterior probability. The vertical, light-grey bar represents the Pleistocene Epoch. Terminals are labeled with taxonomic names, followed by abbreviated localities (CH = China, GR = Greece, GU = Guinea, HU = Hungary, IC = Ivory Coast, ID = Indonesia, IN = India, IR = Iran, JP = Japan, LI = Libya, MA = Malta, MY = Malaysia, PH = Philippines, PM = Peninsular Malaysia, RU = Russia, RY = Ryukyu Islands, TH = Thailand, TW = Taiwan, VT = Vietnam).


Suncus murinus, PH
leucodon
zimmermanni, GR
russula
sicula, MA
obscurior 1, IC
obscurior 2, IC
obscurior 3, GU
batakorum, Palawan, PH
musseri, Sulawesi, ID
sp. 1, Sulawesi, ID
sp. 2, Sulawesi, ID
rhoditis, Sulawesi, ID
elongata, Sulawesi, ID
levicula, Sulawesi, ID
lea, Sulawesi, ID
sp. 3, Sulawesi, ID
Diplomesodon, TU
sp. 4, IN
zarudnyi, IR
shantungensis, TW
mimula, HU
aleksandrisi, LI
sibirica, RU
suaveolens, CH
cf. tanakae, VT
horsfieldii, TH
watasei, RY
fuliginosa 1, VT
fuliginosa 2, PM
maxi, Sumatra, ID
dsinezumi, JP
kurodai, TW
lasiura, RU
attenuata 1, CH
attenuata 2, CH
wuchihensis, CH
paradoxura, Sumatra, ID
lawuana, Java, ID
beccarii 1, Sumatra, ID
lepidura, Sumatra, ID
orientalis, Java, ID
beccarii 2, Sumatra, ID
brunnea, Java, ID
foetida, Borneo, MY
nigripes, Sulawesi, ID
malayana, PM
negligens, Tioman, MY
beatus 1, Leyte, PH
beatus 2, Mindanao, PH
beatus 3, Mindanao, PH
beatus 4, Mindanao, PH
beatus 5, Mindanao, PH
beatus 6, Camiguin, PH
mindorus, Mindoro, PH
mindorus, Sibuyan, PH
negrina, Negros, PH
panayensis, Panay, PH
palawanensis, Palawan, PH
grayi 1, Luzon, PH
grayi halconus, Mindoro, PH
grayi 4, Calayan, PH
grayi 3, Luzon, PH
grayi 2, Luzon, PH
A, B
C
D
E
F
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Millions of Years Ago

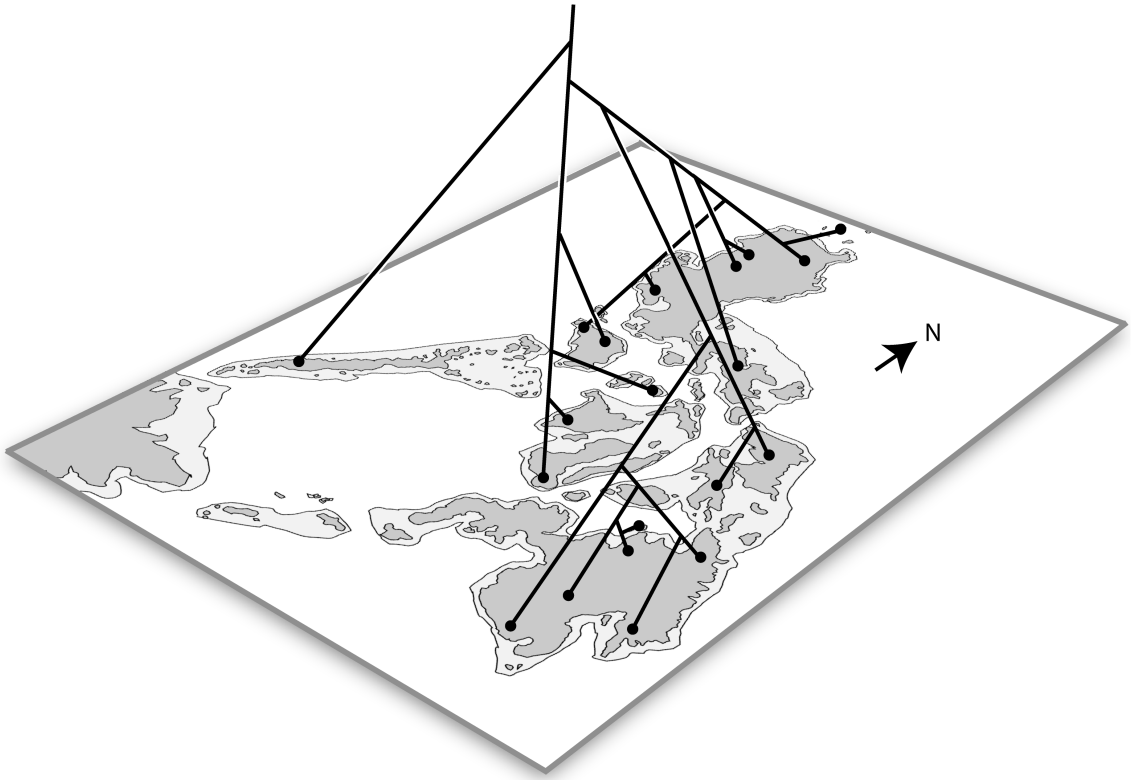


Figure 2.5. Our preferred phylogenetic hypothesis for Philippine *Crocidura*, derived from analyses here and in Esselstyn et al. (2009), and mapped on to Pleistocene geography. Modern islands are shown in medium grey, surrounded by the extent of land during Pleistocene sea-level low stands (–120 m) in light grey. Monophyletic lineages tend to be found on Pleistocene islands more often than would be expected by chance alone.

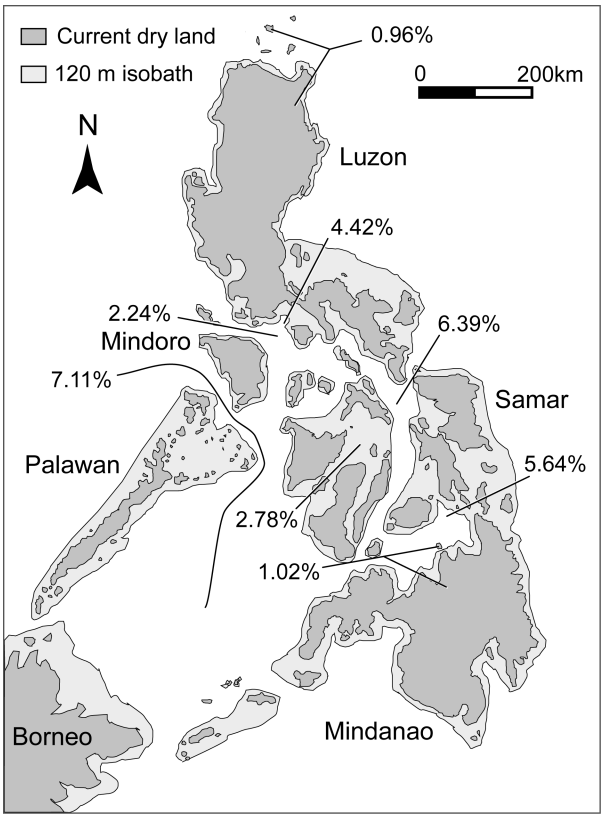


Figure 2.6. Percentages of uncorrected divergence observed in mtDNA sequences across several putative barriers to dispersal and corridors for gene flow, as inferred from the Pleistocene distribution of land.

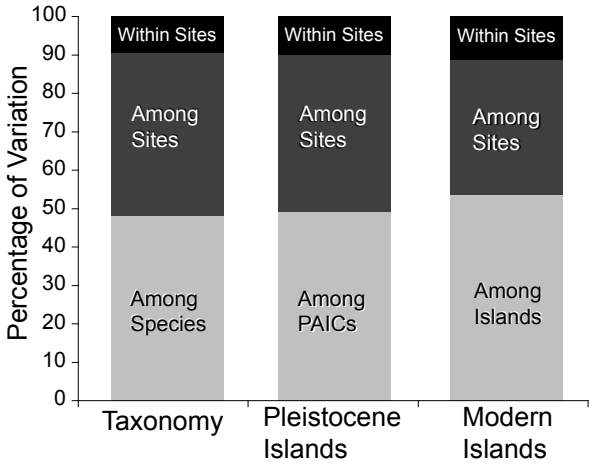


Figure 2.7. Results of three-way AMOVAs. Three hierarchies were used to explore the partitioning of genetic diversity in Philippine *Crocidura*. These included partitioning by taxonomy, by Pleistocene Aggregate Island Complex, and by modern islands.

respectively. In each case, all levels of the three-way AMOVA account for significant proportions of variation ($P < 0.001$).

When samples were analyzed with a PAIC/island/population hierarchy, PAICs explained substantially less genetic diversity in *Crocidura* (29%) than reported for *Haplonycteris* (78%; Roberts 2006b), with both among-island and within-island comparisons accounting for the difference (Fig. 2.8). Some of these differences may be due to the extent and distribution of sampling; however, our iterations on reduced *Crocidura* data sets reveal that the proportions estimated are relatively stable, with modern islands retaining greater explanatory power than PAICs (Fig. 2.9). Removing Luzon from consideration lessens the difference, but modern islands retain slightly more explanatory power (Fig. 2.9).

The AMOVAs further reveal that among island genetic diversity accounts for a substantially larger proportion of variation in *C. beatus* than in *C. grayi* (Fig. 2.10). This is due in part to the deep divergence separating populations on Samar and Leyte from those on Mindanao. These islands are separated by very shallow water and probably were connected as recently as 10K years ago (Siddall et al. 2003; Voris 2000). In contrast, some islands, which have never been connected to any other, hold populations with only shallow genetic divergences separating them from their presumptive source populations. These include populations of *C. grayi* on Calayan Island and *C. beatus* on Camiguin Island, each with uncorrected genetic divergences ≈ 0.01 (Fig. 2.6).

Large islands have the potential to provide opportunities for within-island diversification because of the potential effects of isolation by distance, isolated mountain ranges, and elevational habitat gradients (Evans et al. 2003a; Heaney and Rickart 1990; Steppan et al. 2003; Wright 1950). Mantel tests revealed an effect of isolation by

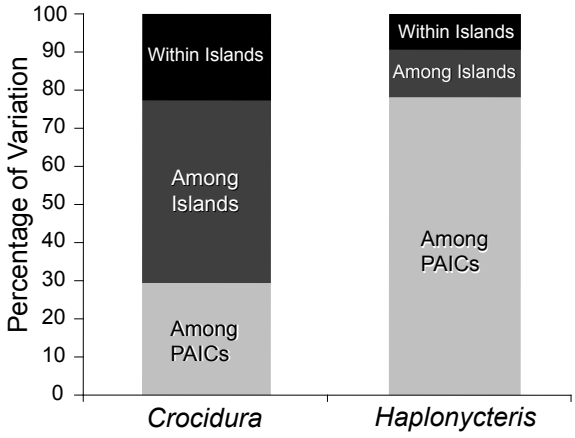


Figure 2.8. Results of three-way AMOVAs comparing the role of Pleistocene Aggregate Island Complexes (PAICs) in structuring genetic diversity in Philippine *Crocidura* and *Haplonycteris* (from Roberts, 2006b).

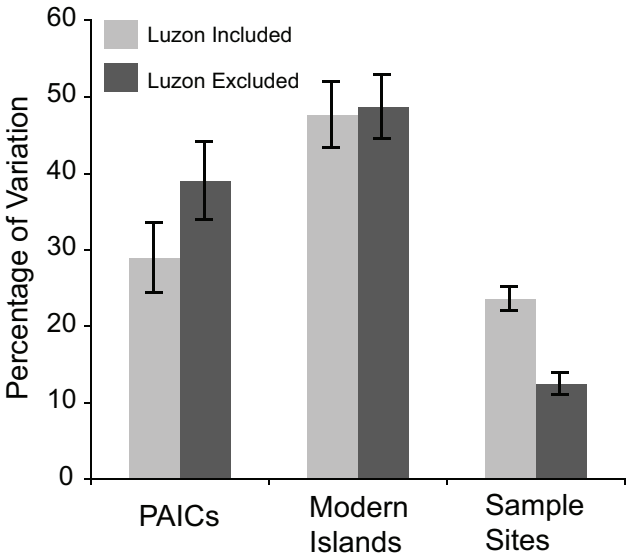


Figure 2.9. Results of jackknifing AMOVAs showing the mean percentage of genetic variation explained by Pleistocene Aggregate Island Complexes (PAICs), islands within PAICs, and sample sites within islands when 36–40% of randomly selected haplotypes have been removed (light grey). All samples from Luzon were removed from the same jackknifed data sets (dark grey). Error bars represent +/– one standard deviation.

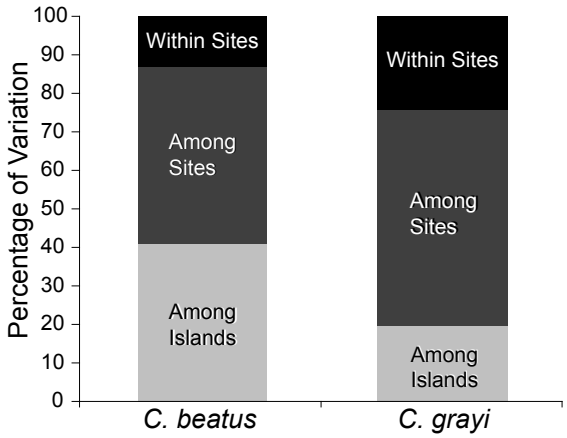


Figure 2.10. Results of three-way AMOVAs showing the role of geography in structuring genetic diversity in *Crocidura beatus* from the Mindanao PAIC and *C. grayi* from the Luzon area.

distance within the widespread species, *C. grayi* and *C. beatus* (Table 2.2). All but one test were significant, indicating that isolation by distance has an effect in most cases. The test with the smallest sample size was insignificant, perhaps due to a lack of statistical power. Island area shows a positive, though not statistically significant relationship with mitochondrial diversity in this data set. This is apparent whether nucleotide diversity is considered across an island (Table 2.1), or at the population level (Fig. 2.11). Regression of these data failed to reveal significant explanatory power ($R^2 = 0.105$, $P = 0.13$), though the positive trend suggests biological significance.

Discussion

Our analyses reveal a complex role for Pleistocene sea-level fluctuations in the diversification of shrews in the Philippines. The topological pattern among island populations is perfectly concordant with PAIC geography (Fig. 2.5), and the probability of this happening by chance alone is small. However, divergences between some island populations within PAICs almost certainly predate the end of the LGM. These divergences may have occurred earlier in the Pleistocene, perhaps associated with previous fluctuations in sea level. Other island populations within PAICs appear to be more closely related and have yet to achieve reciprocal monophyly (e.g., Samar and Leyte populations of *C. beatus*). Given this variation in pattern, the simple assumption that gene flow occurs wherever and whenever dry land is present is probably incorrect for this system and others (e.g., Gorog et al. 2004; Roberts 2006b).

The inference of the timing of speciation events is heavily dependent on calibration strategy. Our estimates based on fossil and secondary calibrations broadly

Table 2.2. Results of Mantel tests on geographic and genetic distances. *P*-values significant at $\alpha \leq 0.05$ are noted in bold.

Species	**Area**	**Z-statistic**	***P*-value**
C. grayi	Luzon Island only	3.797	**0.0034**
	Luzon, Mindoro, Calayan islands	8.258	**< 0.0001**
C. beatus	Mindanao Island only	1.210	0.1270
	Mindanao, Samar, Leyte, Camiguin islands	5.185	**0.0014**

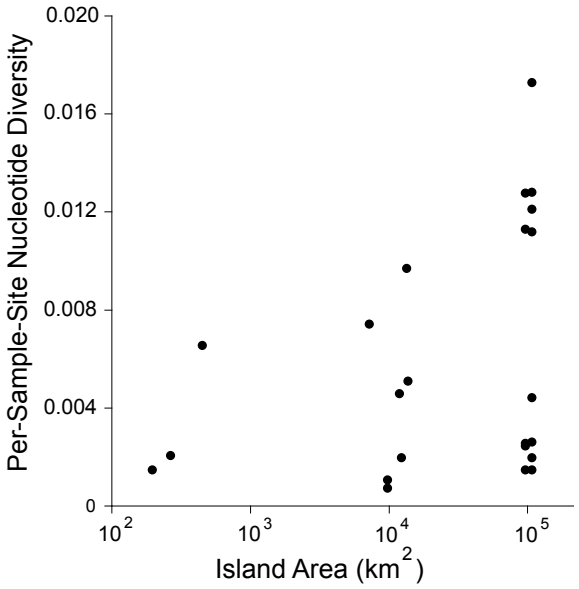


Figure 2.11. Semi-logarithmic plot of within-sample-site nucleotide diversity on island area. Sample sites represented by a single sequence (no estimate of nucleotide diversity) have been removed. A least-squares regression was not significant ($R^2 = 0.105$, $P = 0.13$).

overlap, as do those based on the mean mammalian substitution rate and island age calibrations. The former two produce very young estimates (≤5 My ago) and the latter two very old estimates (≤25 My ago). There is no overlap in 95% highest posterior densities (HPD) between the young and old estimates (Figs. 2.2–2.3), making reconciliation of these two sets of dates challenging. However, we consider it likely that the dates inferred from the fossil and secondary calibrations underestimate the true ages. The earliest known fossil *Crocidura* should be considered a minimum calibration point, but we treated it as a mean age for the origin of the genus in our analysis (Fig. 2.2) because of a lack of potential upper bounds on the age of any node. The secondary calibrations we used also were derived from fossil-calibrated analyses (Dubey et al. 2008) and they produced dates similar to, but slightly older than those from our fossil-calibrated analysis. The per-site substitution rate necessary to generate branch lengths in our fossil-calibrated analysis was much faster (mean = 0.044, 95% HPD = 0.033–0.055 $site^{-1}$ My^{-1}) than the mean mammalian rate (0.0097 $site^{-1}$ My^{-1}). Although there is little basis to choose among potential calibration strategies, we are intrigued by the similarity in age estimates derived from the substitution-rate and island-age calibrated trees. These two analyses produced broadly overlapping HPDs of node ages and are derived from unrelated calibration strategies. However, other than the independence of data sources (or lack thereof), there is little information that might be used to choose between the old and young dates. The use of island ages as calibration points on molecular phylogenies assumes that islands are colonized shortly after they emerge from the sea. Unfortunately, little evidence is available to evaluate this assumption (though see: Brown et al. 2009a; Steppan et al. 2003). We suggest that an analysis testing for rank-correlations between speciation events and island emergences using numerous co-distributed lineages might

shed light on the validity of this assumption. Our combined analysis, which incorporated all potential calibrations and treated the oldest fossil *Crocidura* as a minimum bound, inferred dates that are intermediate, but closer to the young set of dates derived from the fossil- and secondary-calibrated trees. Clearly, any determination of the number of speciation events that took place during the Pleistocene requires better evidence regarding the validity and variance of available calibrations.

Several very shallow divergences separate populations on islands that have never been connected to another landmass (Calayan, Camiguin, and Mindoro) from their closest relatives on Luzon and Mindanao, perhaps suggesting recent colonization of these islands. Although this is not a prediction that could be derived from a PAIC model, occasional over-water colonization events do not necessarily diminish the importance of PAICs in shaping evolutionary history. Colonization of previously uninhabited islands could reasonably be expected to occur throughout history under a PAIC model. We note that all such island populations (i.e., those that are not part of a PAIC: Calayan, Camiguin, Mindoro, and Sibuyan) are most closely related to populations on large, adjacent islands (Esselstyn et al. 2009), suggesting an element of predictability in the colonization process. Divergences between these populations and their putative sources range from <0.01 (Calayan) to >0.06 (Sibuyan). Distinguishing between the effects of distance and PAIC geography will be difficult because islands in close proximity tend to be separated by shallow water.

An effect of geographic distance on genetic diversity also is apparent in the Mantel tests conducted using populations sampled across PAICs and modern islands. The only Mantel test that was not significant was that restricted to Mindanao Island. The number of samples available from the island is limited (Fig. 2.1) and our failure to reject

the null may be due to a lack of statistical power. We note however, that Mindanao Island has a complex geological history that probably includes the accretion of previously isolated islands (Hall 2002). The geography of these palaeo islands may have played a role in generating the substantial genetic diversity seen in some lineages (e.g., Jones and Kennedy 2008; Roberts 2006b) on modern Mindanao and our failure to find a signature of isolation by distance. Island area is known to have a positive correlation with genetic diversity (Nevo 1978; Wright 1931, 1950) and is probably important in shaping patterns of variation in the Philippines, where islands range in area from a few to >100,000 km^2. As expected, the magnitude and variation of within-population (Fig. 2.11) and within-island (Table 2.1) nucleotide diversity rises with increasing island area. The effect is not statistically significant, but the trend suggests biological importance.

AMOVAs revealed a stronger relationship between genetic diversity and modern islands than with PAICs, unlike the pattern noted by Roberts (2006b) in *Haplonycteris*. The limited explanatory power of PAICs in the *Crocidura* data set appears real, as our jackknifing procedures had no effect on the relative proportions explained by PAICs and modern islands (Fig. 2.9). This is quite different from the patterns noted by Roberts (2006b) and Heaney et al. (2005) for forest-dependent bats and a rat, where differences among modern islands within PAICs accounted for little genetic diversity. In another study (Roberts 2006a), three additional lineages of bats were found to have little genetic variation explained by PAIC geography. However these lineages are much less genetically diverse than *Haplonycteris* or Philippine *Crocidura*, suggesting that they are either younger or have experienced greater gene flow across the archipelago.

Jones and Kennedy (2008) concluded that PAICs are not important correlates of genetic variation in four lineages of birds. However, we note that the taxa included in

their study were represented by relatively few samples and that substantial portions of the archipelago were unsampled. Future efforts at testing PAIC models of diversification will be most powerful if they include much denser geographic sampling than is currently available for any taxon. It is unfortunate that all studies to date (this one included) have suffered from limited sampling across the archipelago. Dense sampling might allow one to isolate the effects of sea level fluctuations, distance, and island area.

The role of PAICs in structuring shrew diversity is clearly substantial, but not ubiquitous and probably no more important in explaining geographic patterns of genetic diversity than are modern islands. Clearly there is substantial variation among lineages in the degree of fit of genetic diversity to the expectations of PAIC geography, and ecology may play a role in determining these patterns (Heaney et al. 2005). In the future, densely sampled comparative studies of additional lineages of varying ages, ecologies, and dispersal abilities should provide further insights into the pervasiveness of the 'PAIC effect'. Recent developments in the methods of historical demography (e.g., Drummond et al. 2005) and coalescent-based simulations (e.g., Rosenblum et al. 2007) offer much promise for relating current genetic patterns to past geological and climatic processes. Analyses that combine a comparative approach with tests of explicit *a priori* predictions offer the most potential for untangling the web of potential causes of diversification in this dynamic archipelago.

CHAPTER 3

Colonization of the Philippines from Taiwan: A multilocus test of the biogeographic and phylogenetic relationships of isolated populations of shrews

The Philippine archipelago represents a potential model system for understanding the effects of various geological, climatic, and geographic variables on the diversification trajectories of lineages. Despite this potential, basic features of the evolutionary history of most regional clades, such as the number of times the archipelago was colonized and when and where the colonizations took place, are mostly unknown (but see Brown and Guttman 2002; Esselstyn et al. 2009; Evans et al. 2003a; Jansa et al. 2006; Oliveros and Moyle 2009). Colonization is often the initiation point for evolutionary radiations, adaptive or otherwise (Dobzhansky 1937; Mayr 1942), suggesting a need to understand the process in detail. Knowledge of how, when, and where an invasion took place is crucial to understanding subsequent evolutionary processes in island systems, because this information provides insights into the ages of clades and the extent and tempo of *in situ* diversification.

Several recent phylogenetic and phylogeographic studies have shed light on the process of island colonization (Emerson 2002). Investigations have demonstrated that groups of closely related species may colonize an island group more than once (Carranza et al. 2002; Evans et al. 1999; Gillespie et al. 1994; Rowe et al. 2008), the sources of these colonists may vary (Evans et al. 2003a; Klein and Brown 1994), and continents may be re-invaded by insular lineages (Filardi and Moyle 2005; Nicholson et al. 2005). Some evidence indicates that successful colonization may be dependent on ecological factors, such as pairwise or diffuse competition (Diamond 1975; MacArthur 1972; but

see Simberloff 1978) or on behavioral characters of potential colonists such as the tendency to flock. For instance, in white-eyes (*Zosterops*), flocking may promote colonization by producing large founding populations that have a higher probability of establishing a viable population after arrival (Estoup and Clegg 2003). Unifying the set of factors that potentially influence both the dispersal patterns (e.g., dispersal ability, ocean currents) and likelihood of success upon arrival (e.g., diffuse competition, founding population size) has the potential to provide the basis of models that predict complex patterns of colonization and community assembly. Nevertheless it is apparent from empirical studies that multiple colonizations of individual archipelagos by closely related species are relatively common, having been documented in groups of plants (Díaz-Pérez et al. 2008), invertebrates (Gillespie et al. 1994), and vertebrates (Ruedi et al. 1998).

In the Philippine archipelago, potential colonization routes have long been proposed, including southern routes originating from the Sunda Shelf and Wallacea and a northern route from Taiwan or the Asian mainland, through the Batanes and Babuyan island groups (Fig. 3.1; Dickerson 1928; Wallace 1902). Relatively extensive evidence supports the importance of the southern routes of colonization (e.g., Brown et al. 2009b; Brown and Guttman 2002; Diamond and Gilpin 1983; Esselstyn et al. 2009; Evans et al. 2003a; Heaney 1985, 1986; Jansa et al. 2006; Jones and Kennedy 2008), but the only information known to us that suggests a northern colonization route to have been important is that from the taxonomy of a few bird, mammal, insect, and plant groups (Dickerson 1928) and a recent phylogenetic analysis of Philippine bulbuls (Oliveros and Moyle 2009). Generally, these taxa appear to represent only peripheral invasions of the Philippines, in which lineages colonized the Batanes and/or Babuyan islands, but did not

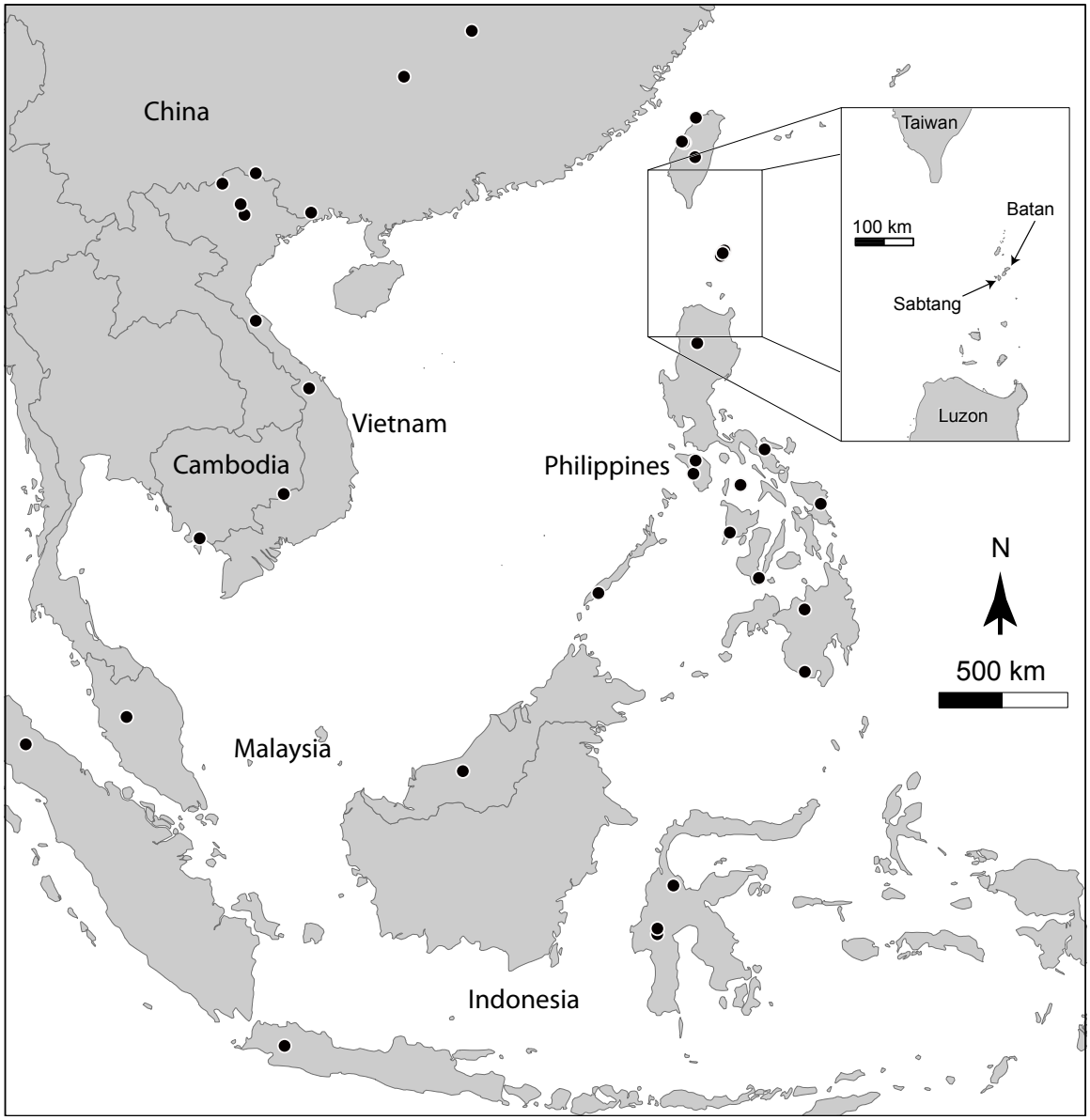


Figure 3.1. Map of Southeast Asia, showing the geographic distribution of samples used in this study. The inset shows the individual islands of the Batanes group, including Batan and Sabtang, and their position relative to potential source pools on Taiwan and Luzon.

succeed in invading the larger islands to the south. Some groups, especially among plants and insects, successfully invaded Luzon, but are limited to the highlands of the northern part of the island (Dickerson 1928). This evidence, of course, is derived from taxonomic associations (excepting Oliveros and Moyle 2009), which often, but not always, reflect evolutionary history. Thus, although the hypothesized northern colonization route has been proposed and some taxonomies suggest that it is an important source of extant diversity in the northern Philippines, it has yet to be tested with explicit estimates of phylogenetic history.

Shrews (Soricomorpha: *Crocidura*) have proven a useful clade for testing a number of biogeographic hypotheses in East Asia, as they are ubiquitous and diverse throughout the region (e.g., Esselstyn and Brown 2009; Motokowa et al. 2005; Ruedi et al. 1998). However, *Crocidura* taxonomy remains complex and somewhat unresolved, as new species and island populations continue to be discovered (Abramov et al. 2008; Hutterer 2007; Jenkins et al. 2007, 2009; Lunde et al. 2004; Ruedi 1995) and molecular evidence has revealed several cases where taxonomy does not fully account for evolutionary history, as inferred from DNA sequence data (Dubey et al. 2008; Esselstyn et al. 2009; Ohdachi et al. 2004).

During the late 1980s, a population of *Crocidura* was discovered on the small, isolated island of Batan, which lies approximately halfway between southern Taiwan and northern Luzon (Fig. 3.1). Heaney and Ruedi (1994) noted the morphological similarity of these specimens from Batan to a series from Taiwan and tentatively placed the newly discovered population within *Crocidura attenuata* (Milne-Edwards 1872), a widespread species then reported from south-central China and Indochina to Taiwan. The Taiwanese

population of *C. attenuata* that Heaney and Ruedi (1994) used in their comparisons originally was described as an endemic species (*Crocidura tanakae* Kuroda 1938), later synonymized with *C. attenuata* (Ellerman and Morrison-Scott 1951; Fang et al. 1997), and then resurrected as a Taiwanese endemic (Fang and Lee 2002). Thus, the Taiwanese specimens Heaney and Ruedi (1994) associated with shrews from Batan Island are now referred to *C. tanakae* (Smith and Xie 2008). Further complicating this history, Esselstyn et al. (2009) tentatively referred a series of specimens from Vietnam and China to *C. tanakae* because they had very similar DNA sequences to specimens from Taiwan. Therefore, it now appears that *C. attenuata* and *C. tanakae* are widespread forms that are morphologically similar, but only distantly related to each other (Smith and Xie 2008; Esselstyn and Brown 2009; Esselstyn et al. 2009).

Recently, fieldwork conducted by C. Oliveros in the Batanes group of islands provided fresh tissue samples of *Crocidura* from Batan Island and revealed the presence of shrews on neighboring Sabtang Island (Fig. 3.1). Here, we use these new specimens to test Heaney and Ruedi's (1994) hypothesis that shrews from Batan Island (and Sabtang Island) are closely related to *C. tanakae* from Taiwan, which implies invasion of the Philippines from the north. We compare this concept to the alternative, in which shrews from Batanes are part of a widespread clade found throughout the more southern parts of the Philippines.

Geological history and faunal diversity of the Batanes Islands

Batan and Sabtang islands are part of a double island arc system, consisting of an eastern and a western chain of islands spanning the Bashi Strait between southern Taiwan and northern Luzon (Yang et al. 1996). The western arc is old, derived from Miocene

volcanic activity, and includes Sabtang Island (Yang et al. 1996). The eastern arc includes Batan Island and is geologically young, with all volcanic activity having occurred after *c*. 2 Ma (Yang et al. 1996). Luzon and Taiwan are substantially older than Batan and Sabtang (Hall 2002). Taiwan was connected repeatedly to the Asian mainland during periods of low sea level, but deep water separates the Batanes Islands from both Taiwan and Luzon (Heaney 1985; Voris 2000). We are not aware of any evidence that might suggest that subsidence has reduced the extent of landmasses in the Bashi Strait. The southern shore of Taiwan and northern shore of Luzon are approximately equidistant from Batan and Sabtang islands (*c*. 200 km).

The mammal fauna of the Batanes Islands is extremely depauperate. Heaney et al. (1998) reported only four species (a shrew and three bats) from the islands. Among these four species, the shrew and one bat (*Pteropus dasymallus*) are considered most closely related to more northerly forms from Taiwan or the Ryukyu Islands (Heaney and Ruedi 1994; Heaney et al. 1998).

Methods

We supplement the multilocus alignment of Esselstyn et al. (2009) with new sequences from shrews sampled from the Batanes Islands, Taiwan, Vietnam, and Cambodia (Fig. 3.1). We include one sequence per species or divergent lineage from published data and all newly generated sequences. The resulting alignments sample all three species known from Taiwan, eight of nine species known from the Philippines, 3–6 (varies among loci) of nine species from Sulawesi, 5–8 of ~25 species from Indochina and the Sunda Shelf, plus several lineages from Indochina and the Philippines that may warrant recognition as distinct species.

We use four single locus alignments to test Heaney and Ruedi's (1994) hypothesis that shrews from Batan (and Sabtang) are more closely related to *C. tanakae* from Taiwan than to any of the species from the more southerly islands of the Philippines. Three of these alignments are derived from fragments of nuclear loci, represented by apolipoprotein B (ApoB), DEAD box Y intron 14 (DBY), and mast cell growth factor introns 5–6 (MCGF). A fourth alignment is a concatenation of the complete sequences of the mitochondrial protein coding genes cytochrome b (cyt b) and NADH dehydrogenase subunit 2 (ND2; Table 1). We sought to make each of the alignments as similar to the others and as complete as possible (in terms of sampled diversity). Accordingly, we excluded some species from the Asian mainland that are available only as published mitochondrial sequences. Nevertheless, all major clades inferred in a previous molecular phylogenetic investigation of Southeast Asian *Crocidura* (Esselstyn et al. 2009) are broadly represented. Taxon sampling in the DBY matrix is less extensive than in the others, because some species were available to us only as female specimens.

DNA isolation, amplification, and sequencing protocols follow Esselstyn et al. (2008, 2009). New sequences of the three nuclear and two mitochondrial genes were generated from specimens from Batan and Sabtang islands, Taiwan, Vietnam, and Cambodia. All new sequences were deposited in GenBank, under accession numbers GU358489–GU358604. Locality data and museum catalog numbers are given in Appendix II.

Each of the four single locus alignments was analysed under Bayesian and maximum likelihood optimality criteria. We used *Suncus murinus* (Linneaus 1766) as the outgroup for all alignments except DBY, where we substituted *Crocidura batakorum* (Hutterer 2007) because of difficulties obtaining sequences of this fragment from *S.*

murinus. Appropriate models of sequence evolution were estimated using Akaike's information criterion (AIC) in MODELTEST 3.7 (Posada and Crandall 1998). If the model favored by AIC was not available in our chosen phylogenetic software, we used the next available, more parameter-rich model. Bayesian analyses were conducted in MrBayes 3.1 (Ronquist and Huelsenbeck 2003) and relied on four runs, each with four chains run for 5×10^6 generations. Samples were drawn from Markov chain Monte Carlo (MCMC) inferences every 1000 generations. We selected an appropriate burn-in based on examination of the trends and distributions of log-likelihoods and parameter values using TRACER 1.4 (Rambaut and Drummond 2007). To assess convergence among MCMC runs, we also examined the correlations of split frequencies among runs in the program Are We There Yet? (AWTY: Nylander et al. 2008).

Maximum likelihood estimates of gene trees were generated in RAxML 7.0 (Stamatakis 2006). One hundred replicate searches were conducted per locus using the default search algorithm. Each search was initiated with a random starting tree. One hundred bootstrap pseudoreplicates were completed and bootstrap support was plotted on the maximum likelihood topology.

To test the hypothesized relationships of *Crocidura* from Batan and Sabtang islands, we employed Bayesian and frequentist approaches to tests of alternative phylogenetic topologies. After completion of phylogenetic inferences, we created constraint trees that included Batan and Sabtang specimens as members of the main Philippine radiation of shrews (including all available Philippine species of *Crocidura* except *C. batakorum*). For the Bayesian approach, we then used PAUP* 4.0b (Swofford 1999) to filter the posterior distribution of trees from each single locus analysis for consistency with the constraint tree. The proportion of trees in the posterior distribution

consistent with the constraint tree provides an estimate of the posterior probability that the hypothesis is true. For the frequentist approach, we employed the Approximately Unbiased (AU) test (Shimodaira 2002). We used RAxML to identify the best tree consistent with the constrained topology (100 searches) before generating per-site log likelihood scores on the best tree under constrained and unconstrained searches. Per-site likelihood scores were then used in CONSEL (Shimodaira and Hasegawa 2001; Shimodaira 2002) to complete the AU test.

Our phylogenetic analyses revealed a very close relationship between shrews from the Batanes Islands and *C. tanakae* from Taiwan. We therefore computed a statistical parsimony network among mitochondrial haplotypes for all available individuals of *C. tanakae*, and several specimens tentatively referred to this species. The network was calculated in TCS 1.21 (Clement et al. 2000) with a 95% confidence limit on haplotype connections and used a matrix of concatenated cyt b and ND2 sequences. We eliminated all missing characters from the mitochondrial matrix for this analysis, reducing the number of nucleotides to 1906. Twenty-four individuals from the Batanes Islands, Taiwan, Vietnam, and China were included in this analysis (Appendix S1). The network is presented as a means of visualizing the mitochondrial diversity found in this lineage, and as an exploratory tool for evaluating the possibility that *C. tanakae* was recently introduced to Batan and Sabtang islands by humans.

Results

Final alignments contain 477–2184 nucleotides and 30–59 ingroup taxa (Table 3.1); each alignment is available on TreeBase under accession S2581. Matrices are mostly complete, with ≤ 7% missing characters. Models of sequence evolution chosen by AIC

Table 3.1. Summary of alignment features and models of sequence evolution estimated with Akaike's information criterion (AIC) and implemented in maximum likelihood (ML) and Bayesian phylogenetic analyses of Southeast Asian *Crocidura*.

Locus	Number of Nucleotides	Number of Ingroup Taxa	AIC Model	ML Model	Bayesian Model
ApoB	577	58	HKY + G	GTR + G	HKY + G
DBY	477	30	K81uf + G	GTR + G	GTR + G
MCGF	635	59	TVM + G	GTR + G	GTR + G
mtDNA	2184	59	GTR + I + G	GTR + I + G	GTR + I + G

for the three nuclear loci were simpler than the available options in MrBayes and RAxML (Table 3.1). In Bayesian phylogenetic inference, all evidence suggests MCMC chains converged in all analyses, as likelihood scores were stable after 2×10^6 generations (or earlier) for all runs and correlations of split frequencies were high. We therefore discarded the first 2×10^6 generations as burn-in for all Bayesian analyses, leaving 12,000 trees (3000 trees per run x 4 runs) in the posterior distribution resulting from each alignment. When pooled among runs, effective sample sizes were estimated at > 1000 for all parameters, in all MCMC analyses.

Shrews from Batan and Sabtang islands are more closely related to *C. tanakae* from Taiwan and other taxa from the Asian mainland than to any species from the Philippines (Figs. 3.2–3.3). All our inferences and topology tests strongly support Heaney and Ruedi's (1994) hypothesis that shrews invaded the northernmost Philippines from Taiwan or the Asian mainland rather than from the more southerly Philippine islands. Although some loci provide greater resolution and support than others, topologies are mostly consistent, and independent analyses of each of the four loci results in the inference of a close relationship among the Batan and Sabtang shrews and *C. tanakae* from Taiwan (Figs. 3.2–3.3). The Bayesian approach to topology tests yields an estimated posterior probability of zero for inclusion of shrews from Batan and Sabtang in the main Philippine clade for all loci and 1.0 for a clade including shrews from Batan and Sabtang and *C. tanakae* from Taiwan, in three of four loci (Table 3.2). Similarly, the AU tests soundly reject any notion that shrews from Batan are a component of the main Philippine radiation, with all locus-specific *P*-values ≤ 0.001 (Table 3.2).

Figure 3.2. Bayesian majority-rule consensus trees of Southeast Asian *Crocidura* derived from sequences of the nuclear genes (a) apolipoprotein B, (b) DEAD box Y intron 14, and (c) mast cell growth factor introns 5–6. The outgroup (*Suncus murinus*) was pruned from (a) and (c) for ease of presentation. Numbers at nodes represent posterior probabilities, followed by maximum likelihood bootstrap support. Taxonomic identities and museum catalogue numbers are given at the terminals. Museum acronyms are defined in Appendix II. Grey boxes indicate the regions from which terminal taxa were collected, with darker grey boxes noting the phylogenetic position of samples from the northern Philippines.

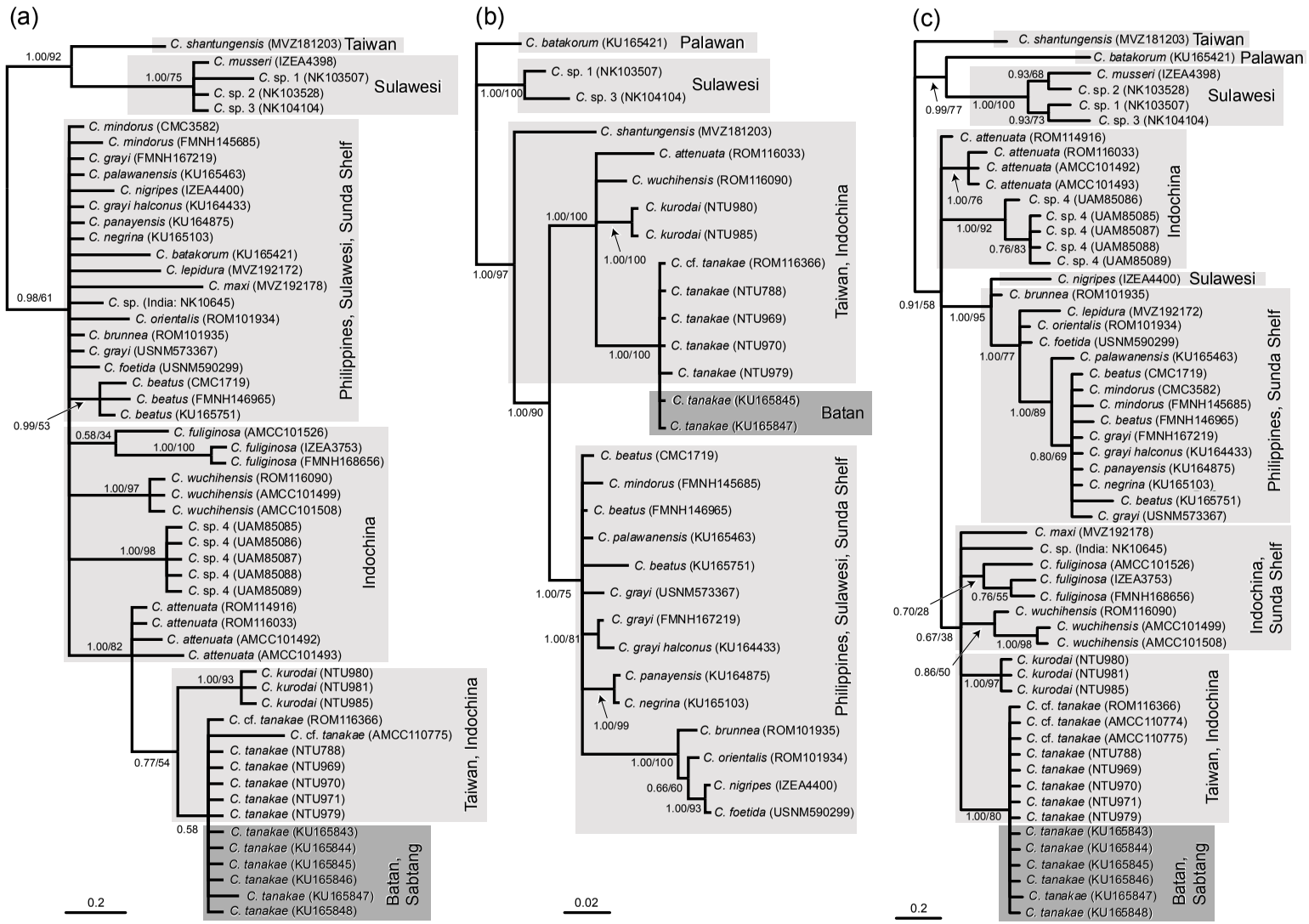
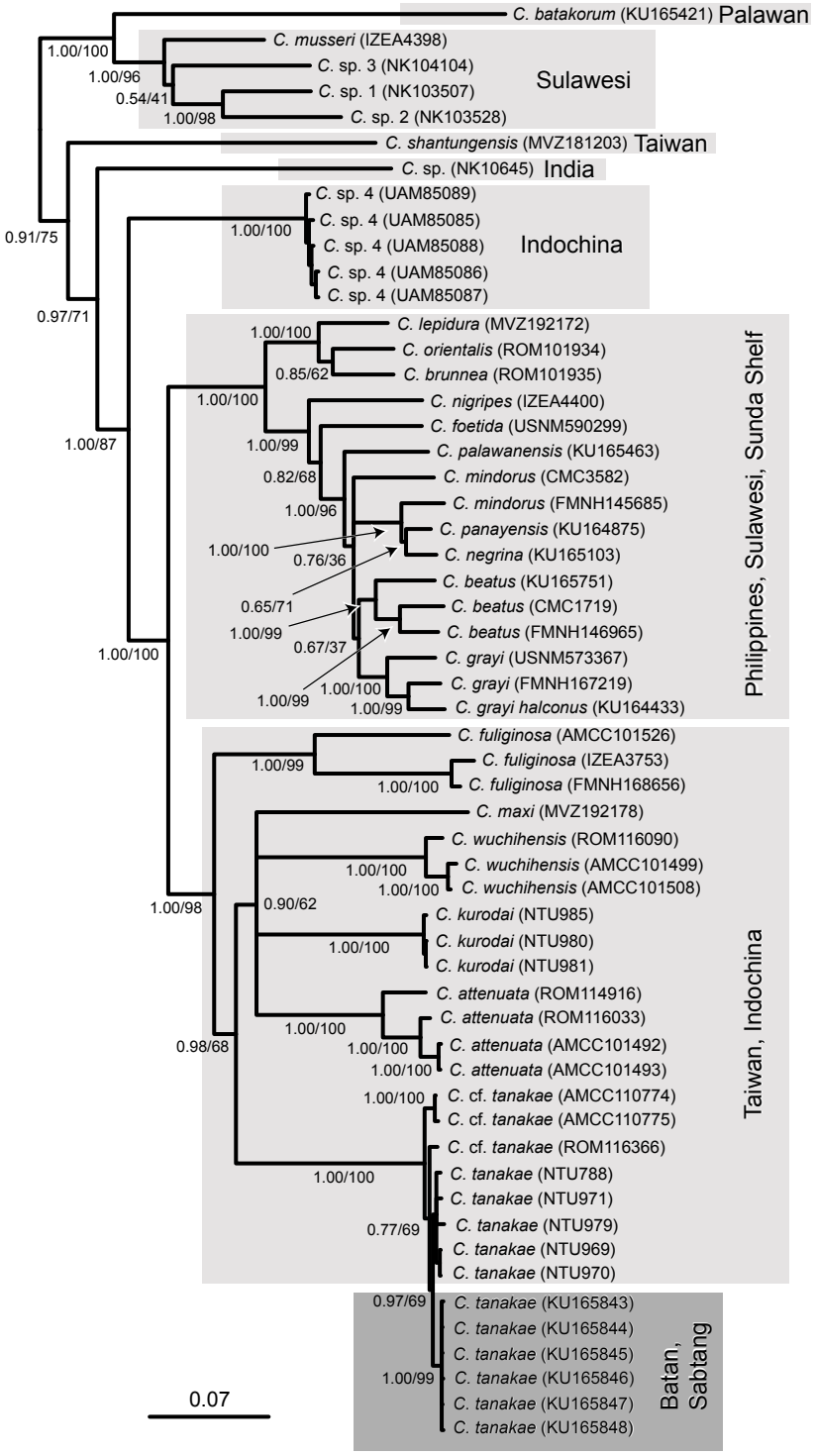

(a)
C. shantungensis (MVZ181203) Taiwan
C. musseri (IZEA4398)
C. sp. 1 (NK103507)
C. sp. 2 (NK103528)
C. sp. 3 (NK104104)
Sulawesi
C. mindorus (CMC3582)
C. mindorus (FMNH145685)
C. grayi (FMNH167219)
C. palawanensis (KU165463)
C. nigripes (IZEA4400)
C. grayi halconus (KU164433)
C. panayensis (KU164875)
C. negrina (KU165103)
C. batakorum (KU165421)
C. lepidura (MVZ192172)
C. maxi (MVZ192178)
C. sp. (India: NK10645)
C. orientalis (ROM101934)
C. brunnea (ROM101935)
C. grayi (USNM573367)
C. foetida (USNM590299)
C. beatus (CMC1719)
C. beatus (FMNH146965)
C. beatus (KU165751)
Philippines, Sulawesi, Sunda Shelf
C. fuliginosa (AMCC101526)
C. fuliginosa (IZEA3753)
C. fuliginosa (FMNH168656)
C. wuchihensis (ROM116090)
C. wuchihensis (AMCC101499)
C. wuchihensis (AMCC101508)
C. sp. 4 (UAM85085)
C. sp. 4 (UAM85086)
C. sp. 4 (UAM85087)
C. sp. 4 (UAM85088)
C. sp. 4 (UAM85089)
C. attenuata (ROM114916)
C. attenuata (ROM116033)
C. attenuata (AMCC101492)
C. attenuata (AMCC101493)
Indochina
C. kurodai (NTU980)
C. kurodai (NTU981)
C. kurodai (NTU985)
C. cf. tanakae (ROM116366)
C. cf. tanakae (AMCC110775)
C. tanakae (NTU788)
C. tanakae (NTU969)
C. tanakae (NTU970)
C. tanakae (NTU971)
C. tanakae (NTU979)
Taiwan, Indochina
C. tanakae (KU165843)
C. tanakae (KU165844)
C. tanakae (KU165845)
C. tanakae (KU165846)
C. tanakae (KU165847)
C. tanakae (KU165848)
Batan, Sabtang
1.00/92
1.00/75
0.98/61
0.99/53
0.58/34
1.00/100
1.00/97
1.00/98
1.00/82
1.00/93
0.77/54
0.58
0.2
(b)
C. batakorum (KU165421) Palawan
C. sp. 1 (NK103507)
C. sp. 3 (NK104104)
Sulawesi
C. shantungensis (MVZ181203)
C. attenuata (ROM116033)
C. wuchihensis (ROM116090)
C. kurodai (NTU980)
C. kurodai (NTU985)
C. cf. tanakae (ROM116366)
C. tanakae (NTU788)
C. tanakae (NTU969)
C. tanakae (NTU970)
C. tanakae (NTU979)
Taiwan, Indochina
C. tanakae (KU165845)
C. tanakae (KU165847)
Batan
C. beatus (CMC1719)
C. mindorus (FMNH145685)
C. beatus (FMNH146965)
C. palawanensis (KU165463)
C. beatus (KU165751)
C. grayi (USNM573367)
C. grayi (FMNH167219)
C. grayi halconus (KU164433)
C. panayensis (KU164875)
C. negrina (KU165103)
C. brunnea (ROM101935)
C. orientalis (ROM101934)
C. nigripes (IZEA4400)
C. foetida (USNM590299)
Philippines, Sulawesi, Sunda Shelf
1.00/100
1.00/97
1.00/100
1.00/100
1.00/90
1.00/75
1.00/81
1.00/99
1.00/100
0.66/60
1.00/93
0.02
(c)
C. shantungensis (MVZ181203) Taiwan
C. batakorum (KU165421) Palawan
C. musseri (IZEA4398)
C. sp. 2 (NK103528)
C. sp. 1 (NK103507)
C. sp. 3 (NK104104)
Sulawesi
C. attenuata (ROM114916)
C. attenuata (ROM116033)
C. attenuata (AMCC101492)
C. attenuata (AMCC101493)
C. sp. 4 (UAM85086)
C. sp. 4 (UAM85085)
C. sp. 4 (UAM85087)
C. sp. 4 (UAM85088)
C. sp. 4 (UAM85089)
Indochina
C. nigripes (IZEA4400) Sulawesi
C. brunnea (ROM101935)
C. lepidura (MVZ192172)
C. orientalis (ROM101934)
C. foetida (USNM590299)
C. palawanensis (KU165463)
C. beatus (CMC1719)
C. mindorus (CMC3582)
C. mindorus (FMNH145685)
C. beatus (FMNH146965)
C. grayi (FMNH167219)
C. grayi halconus (KU164433)
C. panayensis (KU164875)
C. negrina (KU165103)
C. beatus (KU165751)
C. grayi (USNM573367)
Philippines, Sunda Shelf
C. maxi (MVZ192178)
C. sp. (India: NK10645)
C. fuliginosa (AMCC101526)
C. fuliginosa (IZEA3753)
C. fuliginosa (FMNH168656)
C. wuchihensis (ROM116090)
C. wuchihensis (AMCC101499)
C. wuchihensis (AMCC101508)
Indochina, Sunda Shelf
C. kurodai (NTU980)
C. kurodai (NTU981)
C. kurodai (NTU985)
C. cf. tanakae (ROM116366)
C. cf. tanakae (AMCC110774)
C. cf. tanakae (AMCC110775)
C. tanakae (NTU788)
C. tanakae (NTU969)
C. tanakae (NTU970)
C. tanakae (NTU971)
C. tanakae (NTU979)
Taiwan, Indochina
C. tanakae (KU165843)
C. tanakae (KU165844)
C. tanakae (KU165845)
C. tanakae (KU165846)
C. tanakae (KU165847)
C. tanakae (KU165848)
Batan, Sabtang
0.99/77
1.00/100
0.93/68
0.93/73
1.00/76
1.00/92
0.76/83
0.91/58
1.00/95
1.00/77
1.00/89
0.80/69
0.70/28
0.76/55
0.67/38
1.00/98
0.86/50
1.00/97
1.00/80
0.2

Figure 3.3. Bayesian majority-rule consensus tree derived from mitochondrial DNA sequences (cyt *b* and *ND2*) from Southeast Asian *Crocidura*. The outgroup (*Suncus murinus*) was removed for ease of presentation. Numbers at nodes represent posterior probabilities, followed by maximum likelihood bootstrap support. Taxonomic identities and museum catalogue numbers are given at the terminals. Museum acronyms are defined in Appendix II. Grey boxes indicate the regions from which terminal taxa were collected, with darker grey boxes noting the position of samples from the northern Philippines.


C. batakorum (KU165421) Palawan
C. musseri (IZEA4398)
C. sp. 3 (NK104104)
C. sp. 1 (NK103507)
C. sp. 2 (NK103528)
Sulawesi
C. shantungensis (MVZ181203) Taiwan
C. sp. (NK10645) India
C. sp. 4 (UAM85089)
C. sp. 4 (UAM85085)
C. sp. 4 (UAM85088)
C. sp. 4 (UAM85086)
C. sp. 4 (UAM85087)
Indochina
C. lepidura (MVZ192172)
C. orientalis (ROM101934)
C. brunnea (ROM101935)
C. nigripes (IZEA4400)
C. foetida (USNM590299)
C. palawanensis (KU165463)
C. mindorus (CMC3582)
C. mindorus (FMNH145685)
C. panayensis (KU164875)
C. negrina (KU165103)
C. beatus (KU165751)
C. beatus (CMC1719)
C. beatus (FMNH146965)
C. grayi (USNM573367)
C. grayi (FMNH167219)
C. grayi halconus (KU164433)
Philippines, Sulawesi, Sunda Shelf
C. fuliginosa (AMCC101526)
C. fuliginosa (IZEA3753)
C. fuliginosa (FMNH168656)
C. maxi (MVZ192178)
C. wuchihensis (ROM116090)
C. wuchihensis (AMCC101499)
C. wuchihensis (AMCC101508)
C. kurodai (NTU985)
C. kurodai (NTU980)
C. kurodai (NTU981)
C. attenuata (ROM114916)
C. attenuata (ROM116033)
C. attenuata (AMCC101492)
C. attenuata (AMCC101493)
C. cf. tanakae (AMCC110774)
C. cf. tanakae (AMCC110775)
C. cf. tanakae (ROM116366)
C. tanakae (NTU788)
C. tanakae (NTU971)
C. tanakae (NTU979)
C. tanakae (NTU969)
C. tanakae (NTU970)
Taiwan, Indochina
C. tanakae (KU165843)
C. tanakae (KU165844)
C. tanakae (KU165845)
C. tanakae (KU165846)
C. tanakae (KU165847)
C. tanakae (KU165848)
Batan, Sabtang
1.00/100
1.00/96
0.54/41
1.00/98
0.91/75
1.00/100
0.97/71
1.00/100
0.85/62
1.00/100
1.00/87
1.00/99
0.82/68
1.00/96
1.00/100
0.76/36
0.65/71
1.00/99
0.67/37
1.00/100
1.00/99
1.00/100
1.00/99
1.00/99
1.00/100
1.00/98
0.90/62
1.00/100
1.00/100
1.00/100
0.98/68
1.00/100
1.00/100
1.00/100
1.00/100
1.00/100
0.77/69
0.97/69
1.00/99
0.07

We found three mitochondrial haplotypes in the six shrews available from the Batanes Islands (Fig. 3.4). Batan and Sabtang populations are separated from the nearest individual (from Taiwan) by 15 steps in the statistical parsimony network (Fig. 3.4), suggesting they are currently isolated from other populations of *C. tanakae*.

Discussion

Our phylogenetic inferences and topology tests provide conclusive evidence of shrews colonizing the northern Philippines from Taiwan or its immediate vicinity. Although *Crocidura tanakae* successfully invaded the Batanes group of islands, there is no evidence it has established populations south of this area. Shrews were discovered recently in the Babuyan Islands, south of Batanes, but this population is closely related to *Crocidura grayi* from Luzon (Esselstyn et al. 2009), suggesting that *C. tanakae* in the Philippines is restricted to the Batanes Islands.

The extremely close relationship of shrews from Batanes and Taiwan (0.0079 uncorrected *p*-distance in mitochondrial DNA) raises the question of whether the presence of *C. tanakae* on Batan and Sabtang is natural, or the result of human-mediated dispersal. Available evidence is insufficient to allow an explicit test of these alternative hypotheses (natural *versus* human-mediated colonization), but it does permit examination of some plausible scenarios. For example, if shrews colonized Batanes naturally, we expect this population to be established by a very small number of individuals—perhaps even by a single pregnant female. If this were the case, monophyly of single-copy genes (e.g., mitochondrial) would be achieved rapidly, if not instantly (in the case of a single pregnant colonist) within the new population. On the other hand, if human-mediated

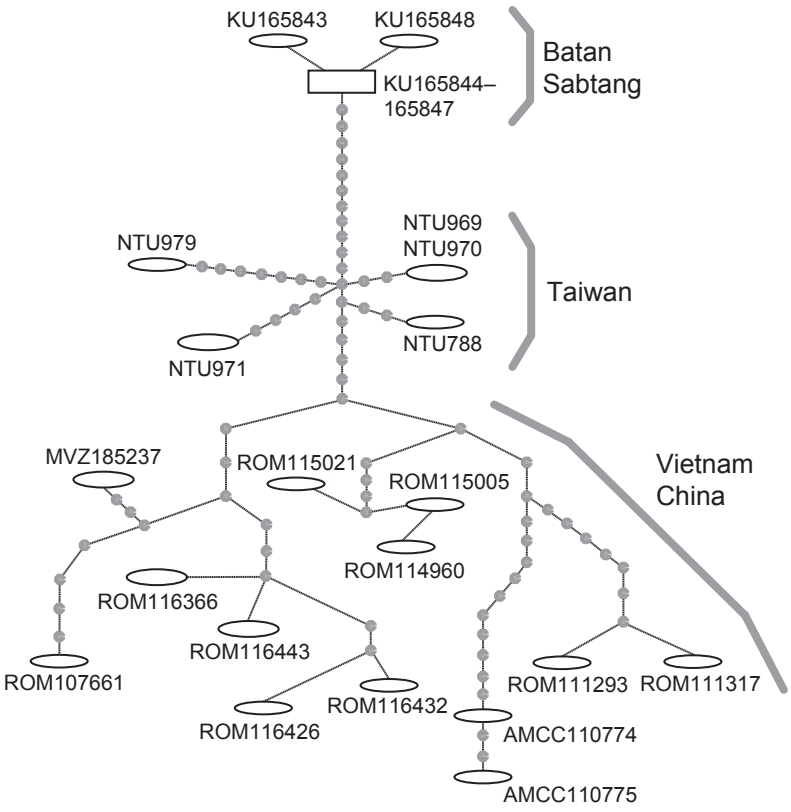


Figure 3.4. Statistical parsimony network of mitochondrial haplotypes in *Crocidura tanakae*, and closely related populations from Vietnam and China tentatively referred to the same species. Grey circles represent individual steps in the network. Museum catalogue numbers are shown at terminals and museum acronyms are defined in Appendix II. Grey brackets indicate the regions from which terminal taxa were collected.

dispersal were responsible for the presence of *C. tanakae* on Batan and Sabtang, we might expect to find multiple, unrelated haplotypes on the islands, and each haplotype might be shared with, or very closely related to haplotypes from, the source population. This pattern would result from regular, or more frequent, arrivals of colonists via an ever-present mechanism (e.g., ship traffic) and would result in our inference of polyphyly among individuals in the exotic population. Among the five specimens from Batan and one from Sabtang, we found three mitochondrial haplotypes, involving two substitutions in *ND2*. Monophyly of these six individuals was strongly supported in Bayesian (estimated posterior probability = 1) and maximum likelihood (bootstrap support = 99) inferences (Fig. 3.3), suggesting a founding colonization by one or a few individuals and implying that the population's presence is natural. Similarly, the parsimony network shows that shrews from the Batanes Islands are isolated by 15 mutational steps from all other individuals of *C. tanakae* (Fig. 3.4), suggesting they are the result of a recent (by geological standards) colonization by a small founding population, and that they are currently isolated from other populations of *C. tanakae*. Finally, if one were willing to assume a molecular clock, even with a fast rate of 0.05 substitutions/site/Myr (Bannikova et al. 2006), the divergence between the Taiwan and Batan populations would date to *c*. 79 ka, well before people began travelling between Taiwan and the northern Philippines *c*. 6 ka (Gray et al. 2009). A recent natural colonization event is not unexpected given that many of the islands between Taiwan and Luzon, including Batan, have origins in the Quaternary (Yang et al. 1996). We presume that most colonizations of oceanic islands by shrews, including this case, are the result of one or a few individuals riding on floating vegetation.

Table 3.2. Results of Bayesian and frequentist tests constraining phylogenetic topology to include shrews from Batan and Sabtang islands to be members of the main Philippine radiation of *Crocidura*, or a member of *C. tanakae* from Taiwan and the Asian mainland. Posterior probabilities (PP) and *P*-values from Approximately Unbiased tests are shown.

Alignment	**PP (Batanes, Taiwan)**	**PP (Batanes, Philippines)**	**P-value (Batanes, Taiwan)**	**P-value (Batanes, Philippines)**
ApoB	0.58	0.00	1.00	5×10^{-6}
DBY	1.00	0.00	1.00	7×10^{-5}
MCGF	1.00	0.00	0.999	0.001
mtDNA	1.00	0.00	1.00	6×10^{-34}

The colonization of oceanic islands by organisms of limited dispersal capacity initiates diversification processes via genetic drift, adaptive radiation, and allopatric speciation. Colonization generates isolation and occasionally puts organisms in places with abundant ecological opportunity (e.g., Harmon et al. 2008). Thus the frequency, directionality, and stochasticity of colonization warrant renewed attention from evolutionary biologists (Cowie and Holland 2006; Heaney 2007). Here, we provide the first compelling test of hypothesized colonization of the Philippines from a northern source by a terrestrial vertebrate. We find that *C. tanakae* colonized the Batanes Islands from Taiwan or the Asian mainland, but this shrew has not succeeded in invading other parts of the Philippine archipelago, where distantly related lineages of *Crocidura* reside (Figs 3.2–3.3). The colonization of Batan and Sabtang by shrews represents the third known instance of invasion of the Philippines by *Crocidura*. Multiple invasions of the country have been noted in other groups, including murid rodents (Jansa et al. 2006), frogs (Brown and Guttman 2002; Evans et al. 2003a), and bulbuls (Oliveros and Moyle 2009). An emerging pattern is that some invasions result in substantial diversification while others appear not to generate speciation events (or extinction eliminates the evidence). The data presented here and by Esselstyn et al. (2009) strongly suggest that *Crocidura* invaded the Philippines once from the Sunda Shelf, once from Wallacea or the Sunda Shelf, and once from the Taiwan region. However, only one of these colonization events (the one from the Sunda Shelf) led to widespread ubiquity and *in situ* speciation.

Several recent studies suggest a need to recognize an inherent complexity in patterns of island colonization over geological time scales, in which isolated archipelagos may be invaded multiple times from multiple sources by groups of closely related

species. Multiple invasions have been noted in several lineages in Southeast Asia (Brown and Guttman 2002; Evans et al. 2003a; Jansa et al. 2006; Oliveros and Moyle 2009), as well as in other archipelagos, including the Hawaiian Islands, West Indies, and Macaronesia (Díaz-Pérez et al. 2008; Gillespie et al. 1994; Klein and Brown 1994). Successful colonization requires both the dispersal of propagules to an island, and reproduction after the journey. Good colonizers will thus possess traits that yield a tendency to disperse and a capacity to reproduce upon arrival. Although shrews do not possess features that clearly define them as good colonizers, their ubiquity on islands throughout much of Southeast Asia indicates that they have been quite successful at establishing populations on oceanic islands. The present distribution of *Crocidura* in the oceanic Philippines is highly regular; most islands that have been adequately surveyed (16 islands with records of *Crocidura*: Esselstyn et al. 2009; Heaney and Ruedi 1994; Heaney et al. 1998; Rickart et al. 1993) hold a single species of *Crocidura*, suggesting a possible role for competition in preventing secondary colonization (e.g., MacArthur 1972). This distribution implies that shrews are good at colonizing islands that lack shrews, but they may struggle to persist after arrival on an island that is already inhabited by a closely related species. The two exceptions to the one species per island pattern (Mindoro and Mindanao islands) are each inhabited by two species. In both cases, one species appears to be a restricted range, high-elevation specialist, whereas the other is widespread and common throughout the island, perhaps limiting the interaction between these species. If pairwise competition does exclude potential colonists, then dispersal events to neighboring islands may frequently fail to establish populations, and inter-island dispersal may be far more common than is generally appreciated.

CHAPTER 4

Does competitive exclusion prevent inter-island colonization by shrews? An integrative approach to testing for effects of species interactions on diversification

Theory predicts that closely related species cannot coexist until they have diverged sufficiently in ecologically important traits (Darwin 1859; Gause 1934; Grinnell 1904; Hutchinson 1957; Lack 1947). Ecological differentiation may happen rapidly in clades undergoing adaptive radiation (Schluter 2000), but much of biological diversity probably results from speciation across geographic barriers, with relatively little attendant divergence in ecologically important traits (Jordan 1908; Mayr 1963; Peterson et al. 1999; Wiens 2004). If so, competition may result when closely related, initially isolated species come into contact, and these interspecific interactions may result in competitive exclusion.

Such coevolutionary thinking was endorsed enthusiastically until the 1970s (e.g., Gause 1934; Hardin 1960), but subsequently has been treated with more caution (Connell 1980; Gould and Lewontin 1979; Grant 1986; Simberloff 1978). Nevertheless, studies continue to document patterns consistent with competition playing a role in community structure (e.g., Cooper et al. 2008; Diamond 1975; Gurevitch et al. 1992; Moen and Wiens 2009; Passarge et al. 2006). Although most authors acknowledge some role of competition in shaping communities under particular circumstances, questions remain as to its potency, pervasiveness, and results. For instance, Grant (1986) questioned the role of competition in stable environments, but considered it to have strong effects at points of ecological stress, suggesting a need to consider spatial and temporal scales in studies of competition.

Unfortunately, competition is difficult to demonstrate or refute in empirical studies of free-living organisms. Much of the argument for competitive exclusion therefore derives from theoretical treatments (Neill et al. 2009), empirical microcosm studies (Chan et al. 1985; Dib et al. 2008; Passarge et al. 2006), and correlational studies of patterns of co-occurrence among natural communities (Cavendar-Bares 2004; Cooper et al. 2008; Diamond et al. 1989). Thus, the pervasive observation of sister species with abutting peripatric distributions represents a classic form of evidence for competitive exclusion (e.g., Diamond 1986; Jordan 1908). However, other processes generate the same pattern (den Boer 1986; Simberloff 1978). For example, both vicariant speciation and competitive exclusion can produce adjacent ranges for closely related species, making it difficult to distinguish among potential underlying mechanisms. Nevertheless, if competitive exclusion is the underlying mechanism, then the competing species *must* occupy similar ecological space. However, sister species isolated by vicariant events or dispersal over barriers may also be ecologically similar, as expected under niche conservatism. Comparisons of ecological dimensions occupied by closely related, peripatric species may therefore sometimes lead to insights regarding competition's role in shaping distributions, but in other cases, it will be uninformative. Until very recently, techniques for quantifying ecological similarity were limited, and primarily anecdotal (den Boer 1986). However, with the advent of ecological niche modeling and associated statistical tests, a course-resolution, objective means of assessing ecological similarity is now available (Anderson et al. 2002; Peterson et al. 2002; Warren et al. 2008).

Most discussion of niche conservatism centers on the Grinnellian variety (e.g., Peterson et al. 1999), emphasizing the environmental dimensions occupied by a species. This concept of niche is useful from a practical standpoint because of the availability of

environmental data, and we focus on it here. If Grinnellian niches are conserved over evolutionary time scales and niche similarity results in competition, then secondary colonization of habitats occupied by closely related species should lead to either local extinction of one species (exclusion) or character displacement in some ecologically significant character that lessens competition and permits coexistence (e.g., Pritchard and Schluter 2001). If so, then within clades that generally speciate across geographic barriers, co-occurring species are expected, on average, to be more distantly related and more different ecologically from one another than expected under a model of random draws from the regional species pool. In other words, if this competition thinking is correct, sympatric species should be overdispersed on the phylogeny and in ecological dimensions (Cavendar-Bares et al. 2004; Cooper et al. 2008; Webb et al. 2002).

Here, we combine a variety of approaches to explore the potential role of competitive exclusion in limiting inter-island colonization, and hence speciation, in a group of shrews (genus *Crocidura*) endemic to the Philippine archipelago. We employ ecological niche modeling, analyses of phylogenetic and ecomorphological dispersion, and simulations of inter-island colonization to develop an integrative understanding of potential constraints on diversification.

Geographic Setting

The Philippines has a remarkably complex geological history, in which a combination of volcanic activity, subduction, and island accretion altered the distribution of land dramatically over the history of the archipelago (ca. the last 30 My). Detailed models and descriptions of the evolution of the archipelago are provided by Hall (1998, 2002) and Yumul et al. (2009). Discussion of many of the biologically relevant events can be

found in Heaney et al. (1998, 2005, 2006), Evans et al. (2003a), Brown and Diesmos (2009), Esselstyn et al. (in review), and papers cited therein.

However, with regard to the relatively recent ecological and evolutionary processes considered here, the most relevant aspect of the geographic history of the archipelago is that of sea-level fluctuations, and the resulting aggregation of islands currently separated by shallow seas. Because the large complex islands of the Philippines are the product of accretion of past islands (Hall 1998, 2002; Yumul et al. 2009), geologically driven vicariance is largely absent from the Philippines. However, sea levels have fluctuated widely since the late Pliocene and throughout the Pleistocene. During periods of glacial maxima, global sea levels were reduced by up to *ca*. 120 m (Bintanja et al. 2005; Miller et al. 2005), resulting in the repeated connection and isolation of modern islands in the Philippines (Dickerson 1928; Heaney 1985; Inger 1954; Voris 2000). When sea levels were low, six major islands were formed, here termed greater Luzon, Mindanao, Mindoro, Negros–Panay, Palawan, and Sulu (Fig. 4.1). We refer to these as Pleistocene Aggregate Island Complexes (PAICs: Brown and Diesmos 2002). Presumably, the repeated connections allowed for dispersal of plants and animals over these land bridges. However, phylogeographic evidence suggests the effect has not been universal (Brown and Guttman 2002; Evans et al. 2003a; Heaney et al. 2005; Roberts 2006a, b; Esselstyn and Brown 2009; Siler et al. 2010).

Distributional Patterns of Shrews in the Philippines

Crocidura shrews are widely distributed in the Philippines; they have been documented on all but one of the PAICs (Sulu) and on a few oceanic islands (Esselstyn et al. 2009; Heaney and Ruedi 1994; Heaney et al. 1998; Hutterer 2007; Fig. 4.1; Table 4.1).

Phylogenetic relationships among the 10 species currently recognized from the country are increasingly well resolved (Esselstyn et al. 2009; Esselstyn and Oliveros 2010; Esselstyn and Goodman, in review). One species, *C. tanakae*, occurs only at the very northern extremity of the Philippines, in the Batanes Islands—this species is closely related to populations from Taiwan and the Asian mainland, but only a distant relative of other Philippine *Crocidura* (Esselstyn and Oliveros 2010); as it is part of a distinct biogeographic setting and species pool, we exclude it from further consideration. Among the remaining nine species, at least seven are members of an endemic Philippine clade that occurs throughout the country, from Calayan in the north, to Palawan, Balabac, and Mindanao in the south (Esselstyn et al. 2009; Fig. 4.1; Table 4.1). One species (*C. grandis*) has not been recorded in over a century and is known only from the holotype (Miller 1910), but likely is a member of the endemic Philippine clade (Heaney and Ruedi 1994). Another species (*C. batakorum*), occurring on Palawan, is most closely related to an endemic Sulawesian radiation of *Crocidura* (Esselstyn et al. 2009). Among the nine species we consider here, most are endemic to a single PAIC or oceanic island (Fig. 4.1; Table 4.1). The two exceptions are *C. grayi*, which occurs on Greater Luzon, but also on Mindoro and Calayan islands, both of which are isolated by deep water. The other is *C. beatus*, which occurs on the islands of Greater Mindanao, but also on Camiguin Sur, a small, young volcanic island that has remained isolated throughout its existence (Heaney and Tabaranza 2006). Thus, most islands in the Philippines hold single species of *Crocidura*, but two species are found on the islands of Mindanao (*C. beatus* and *C. grandis*), Mindoro (*C. grayi* and *C. mindorus*), and Palawan (*C. batakorum* and *C. palawanensis*: Fig. 4.1; Table 4.1). We generally refer to species with vouchered localities from the same island, as sympatric.

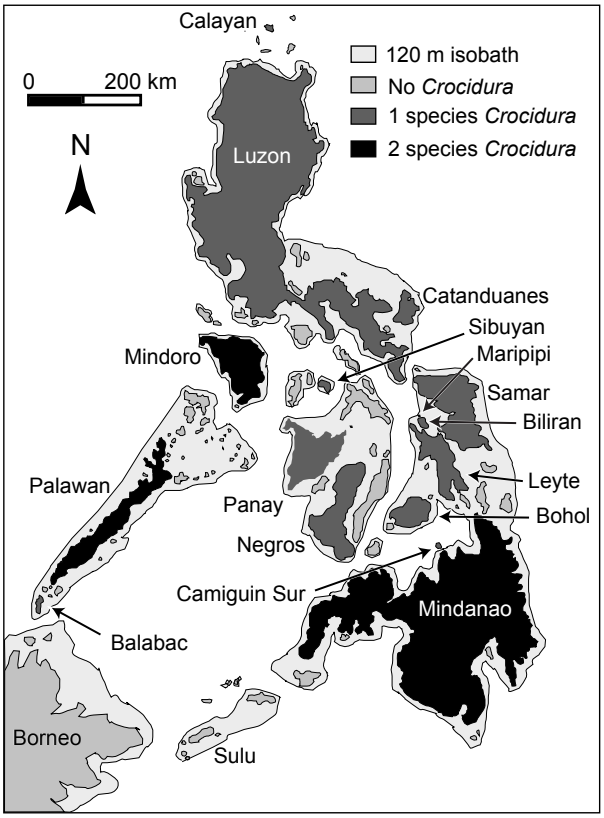


Figure 4.1. Map of the Philippines showing the extent of land during Pleistocene sea-level low-stands in light gray. Modern islands are shaded according to their shrew diversity, with islands lacking *Crocidura* records as medium gray, those with 1 documented species of *Crocidura* as dark gray, and those with two documented species are black. Borneo is excluded from the diversity-shading scheme. Species recorded from each island are given in Table 4.1.

Methods

Modeling Potentially Suitable Ecological Space

Most species of Philippine *Crocidura* are known from a few localities. Two species (*C. grayi* and *C. beatus*), however, have moderately wide geographic distributions, each with at least 36 spatially unique, vouchered localities (Esselstyn and Brown 2009). To characterize ecological niches of Philippine *Crocidura*, we used all known sampling localities to generate ecological niche models (ENMs) for *C. grayi* and *C. beatus* using Maxent 3.3.2 (Phillips et al. 2006).

Maxent uses an algorithm based on the principle of maximum entropy. The product of the algorithm is a probability distribution from the environmental and occurrence data in which the best explanation is that which shows the broadest (i.e., most spread out) probability distribution. Maxent fits this distribution subject to particular constraints, in this case, environmental values associated with collection localities. The logistic output is considered by some an analogue of the probability of species occurrence in a Bayesian context (Philips and Dudík 2008). To convert the resulting map of continuous probabilities to a predicted presence/absence map, we used the lowest probability in our training occurrence data as a threshold, where lower probabilities were considered absence (Pearson et al. 2007).

We generated ENMs using vouchered localities (*C. grayi* = 51 [includes *halconus*], *C. beatus* = 36) and raster GIS layers summarizing relevant climate parameters. Climate data consisted of seven WorldClim layers (Hijmans et al. 2005) that represent variation in precipitation and temperature (annual mean temperature, mean diurnal temperature range, maximum temperature of warmest month, minimum

Table 4.1. Distribution of shrews (*Crocidura*) in the Philippines (excluding the Batanes Islands). Island areas are drawn from Heaney et al. (2002) and Allen et al. (2006). The Pleistocene Island column indicates to which Pleistocene Aggregate Island Complex the island belongs, if any. GenBank accession numbers are given for populations included in the test of phylogenetic dispersion.

Species	**Island**	**Area (km^2)**	**Pleistocene Island**	**GenBank Accessions: CytB / ND2**
Crocidura batakorum	Palawan	11,785	Palawan	FJ813976 / FJ814541
Crocidura beatus	Biliran	498	Mindanao	
	Bohol	3864	Mindanao	
	Camiguin Sur	249	--	FJ813985 / FJ814550
	Leyte	7213	Mindanao	
	Maripipi	22	Mindanao	
	Mindanao	96,467	Mindanao	FJ813844 / FJ814410
	Samar	13,429	Mindanao	
Crocidura grandis	Mindanao	96,467	Mindanao	
Crocidura grayi	Calayan	196	--	FJ813930 / FJ814495
	Catanduanes	1513	Luzon	
	Luzon	107,170	Luzon	FJ813850 / FJ814416
	Mindoro	9735	Mindoro	FJ813932 / FJ814497
Crocidura mindorus	Mindoro	9735	Mindoro	FJ813840 / FJ814406
Crocidura negrina	Negros	13,670	Negros–Panay	FJ813957 / FJ814522
Crocidura sp. nov.	Sibuyan	449	--	FJ813841 / FJ814407
Crocidura palawanensis	Balabac	306	Palawan	
	Palawan	11,785	Palawan	FJ813978 / FJ814543
Crocidura panayensis	Panay	12,300	Negros–Panay	FJ813945 / FJ814509

temperature of coldest month, annual precipitation, precipitation of wettest month, and precipitation of driest month), and are generally uncorrelated with each other (Jiménez-Valverde et al. 2009).

We plotted vouchered occurrence points and regions that could be reasonably assumed to have been available for colonization by the species, as "M" in the "BAM" framework of Soberón (2007) and Soberón and Peterson (2005). The BAM concept is best visualized as a Venn diagram, in which an organism's geographic distribution is recognized as the intersection of the biotic (B), abiotic (A), and movement (M) components of the organism's niche and history. The movement component (M) is intended to represent areas the species has explored during its history. For the purpose of this study, M was defined as all islands with a vouchered record of the species, plus all islands united with them during Pleistocene glacial maxima. For *C. grayi*, this area included greater Luzon and Mindoro, as well as Calayan Island (Fig. 1). The M area for *C. beatus* included Greater Mindanao and Camiguin Island (Fig. 1).

We generated ENMs for each species with current climate data, drawn from their respective M areas. These ENMs were then projected onto the entire Philippine archipelago and northern Borneo using current climate data and Pleistocene reconstructions of environmental layers representing the last glacial maximum (20 Kya) and last interglacial (135 Kya: Otto-Bliesner et al. 2006). To the latter, we applied the threshold defined from the current climate data (Hijmans et al. 2005).

As a test of the hypothesis that *Crocidura beatus* and *C. grayi* are ecologically similar, and therefore potential competitors, we calculated the niche overlap metrics, Hoellinger's based I and Schoener's D, for the previously described, thresholded ENMs

generated under the current climate regime. Niche similarity was evaluated using a variant of the background similarity test of Warren et al. (2008), as implemented in ENM Tools. We generated random occurrence points (51 for *C. grayi* and 36 for *C. beatus*) within the area of M for one of the two species being compared. ENMs were generated using Maxent 3.3.2 from these points (as above), thresholded with the minimum presence value, and compared to the empirical thresholded ENM of the other species to calculate the overlap metrics I and D. We placed observed overlap values in the resulting null distributions of I and D and calculated one-tailed *P*-values, testing only for non-similarity of ecological niches.

Testing for Phylogenetic Overdispersion

We used previously published mitochondrial DNA sequence data to infer an ultrametric tree for eight of the nine species (*C. grandis* is unavailable) of Philippine *Crocidura* considered here (Esselstyn et al. 2009). A concatenated character matrix of Cytochrome b and NADH dehydrogenase subunit 2 (ND2) was used (2184 nucleotides). The matrix is nearly complete, with only 10 characters missing from the 3' end of ND2 in *C. mindorus*. A single individual of each species, from each of the PAICs on which it occurs, was used. For the eight species sampled, a total of 11 individuals were included (Table 4.1), comprised of three representatives of *C. grayi* (one each from the islands of Luzon, Mindoro, and Calayan), two of *C. beatus* (one each from the islands of Mindanao and Camiguin), and one of each of the remaining species. In other words, we treat populations on islands separated by deep ocean channels, which have never been connected to one another (Heaney 1985; Voris 2000), as species.

Phylogenetic topology and branch lengths were inferred in a Bayesian framework using BEAST v1.5.3 (Drummond and Rambaut 2007). Six independent runs of 5 million generations were completed using a GTR + G model of sequence evolution and Yule speciation prior. Parameters were sampled every 2000 generations and the initial 300,000 generations of each run were discarded as burn-in, leaving 15,000 trees in the posterior distribution. To evaluate convergence among MCMC analyses, trends and distributions of parameters, including the likelihood score, were examined in Tracer v1.4 (Rambaut and Drummond 2007). The posterior distribution of trees was summarized on a maximum clade credibility tree with branch lengths presented as median heights.

Pairwise patristic distances (i.e., sums of branch lengths separating two terminals) were calculated between all terminals using the DendroPy phylogenetic library (Sukumaran and Holder 2009). We then calculated the means of pairwise patristic distances among sympatric species pairs and among allopatric species pairs, and the difference between the two as

$$\Delta Patristic = \bar{X}_S - \bar{X}_A,$$

where $\bar{X}_S$ is the mean of pairwise patristic distances separating sympatric species and $\bar{X}_A$ is the mean of pairwise patristic distances separating allopatric species. The value of $\Delta Patristic$ provides a measure of phylogenetic dispersion. If $\Delta Patristic$ is positive, sympatric species are distant relatives, indicating the presence of a sympatry threshold and competitive exclusion, or alternatively, allopatric speciation resulting from inter-island colonization. If $\Delta Patristic$ is negative, this indicates either habitat filtering, in

which closely related species tend to occur sympatrically because they have similar ecological needs, or within-island speciation. Because no tissue samples of *C. grandis* are available, this test incorporated only two sympatric species pairs (*C. grayi* and *C. mindorus* from Mindoro and *C. batakorum* and *C. palawanensis* from Palawan). As a means of measuring statistical significance, we recalculated Δ*Patristic* 2000 times on the empirical matrix of distances, with sympatry (two species pairs) randomized among the terminals. This approach is similar to the widely used Net Relatedness Index (Webb 2000; Webb et al. 2002), but allows us to calculate a single measure of dispersion for multiple two-species communities.

Testing for Overdispersion in Body Size

Body size represents an important ecomorphological trait in shrews; communities of sympatric species are often noted for their highly regular distributions of body size (Kirkland 1991). Here, we use the length of the skull as a proxy for body size, and test for size overdispersion among sympatric species. JAE measured the greatest length of skulls from the posterior margin of the occipital condyles to the anterior margin of the incisors (condylo-incisive length), using digital calipers precise to the nearest 0.01 mm. Only adult specimens, as judged by complete fusion between the basioccipital and basisphenoid bones and fully erupted molars, were measured. The average skull length was calculated for each species or island population, and pairwise differences in mean skull length were calculated between all species/island populations. As with the phylogenetic analysis, we included representatives of each species from all permanently isolated islands on which it occurs. Thus, all of the island populations included in the phylogenetic analysis are represented here. In addition, we include the holotype of

Crocidura grandis, resulting in the representation of all known species of *Crocidura* from our focal area and inclusion of all three sympatric species pairs (*C. palawanensis* and *C. batakorum* from Palawan, *C. grandis* and *C. beatus* from Mindanao, and *C. grayi* and *C. mindorus* from Mindoro). The test statistic for body-size dispersion was calculated as

$$\Delta Size = \bar{Y}_S - \bar{Y}_A,$$

where $\bar{Y}_S$ is the mean of differences in body size among sympatric species pairs and $\bar{Y}_A$ is the mean of differences in body size among allopatric species pairs. A null distribution for $\Delta Size$ was generated by randomizing sympatry (three pairs) among the species and recalculating $\Delta Size$ 2000 times.

We also tested for phylogenetic signal in body size using Pagel's lambda (Pagel 1999). Likelihood scores for untransformed and transformed trees were calculated in the R package GEIGER (Harmon et al. 2008) and significance was evaluated with a likelihood ratio test. The result was compared to a chi-square distribution with one degree of freedom.

Simulating the Process of Island Colonization

We simulated the process of island colonization to determine whether the geographic distribution of Philippine *Crocidura* could be generated by the minimum number of colonization events necessary to explain their distribution. In other words, we asked whether competitive exclusion might have caused failure of past inter-island dispersal events after arrival of potential propagules on an occupied island. A single island was

randomly selected as the first island with a shrew population. This seeding event was not counted as a colonization event. From there, colonization events occurred one at a time with the source population selected at random from among occupied islands. The recipient island was selected among all the islands with a probability inversely proportional to a measure of distance from the source. The simulation was run with two distinct probabilities for selecting the recipient island: (1) the probability of colonizing a particular island was inversely proportional to its minimum inter-shore distance from the source island, and (2) this probability was the inverse of the distance squared. We adopted the second approach to account for our expectation that long-distance colonization in shrews should be much rarer than short-distance colonization; squaring the distance results in much lower probability for long-distance colonization. For this simulation, we treated island groups united during Pleistocene sea-level low-stands as single islands. Minimum distances among these PAICs were measured using Google Earth and were taken between the shores of the nearest modern islands with an area ≥100 km^2 within each Pleistocene island. Because uncertainty exists as to exactly how many islands have extant populations of *Crocidura*, we adopted three geographic scopes in our simulations. These included scenarios where 8 of 14 islands, 8 of 9 islands, and 5 of 6 islands had been colonized. In the 14-island scenario, all 5 Pleistocene islands and 3 oceanic islands with shrew records, plus the largest PAIC lacking a shrew record (Sulu) were included. Also included were all oceanic islands with an area ≥100 km^2 and records of at least three native mammal species (Heaney et al. 2002, 2010; Oliveros and Esselstyn, unpubl.). In other words, we included oceanic islands that are sufficiently large that they can be expected to hold shrew populations and that have been the subject of at least cursory biodiversity inventories (i.e., Babuyan Claro, Camiguin Norte, Lubang,

Siquijor, and Tablas). In the 9-island scenario, all 5 Pleistocene islands and 3 oceanic islands with shrew records, plus the largest PAIC lacking a shrew record (Sulu) were included. In the 6-island scenario, only the PAICs were included, leaving out the oceanic islands of Camiguin, Calayan, and Sibuyan (Fig. 4.1). Under each scenario, the total number of colonization events was recorded during each of 10,000 replicates.

Results

Modeling Potentially Suitable Ecological Space

Ecological niche models estimate broad geographic overlap in the potentially suitable ecological spaces for *Crocidura beatus* and *C. grayi* (Fig. 4.2). Both species are predicted to find suitable climatic space across much of the Philippines and northern Borneo under current climate conditions, and during the Last Glacial Maximum and Last Interglacial conditions. Tests of niche overlap failed to reject the null hypothesis that *C. beatus* and *C. grayi* have similar niches, using both metrics of similarity and with independent randomizations of each species' occurrence data (Table 4.2).

Phylogenetic Dispersion

Phylogenetic inferences were consistent across six independent Markov Chain Monte Carlo analyses. Examination of trends in log-likelihood scores and other parameters suggest that all six runs converged within the first 300,000 generations. Effective sample sizes for all parameters were greater than 200, and most were greater than 1000. The topology inferred here (Fig. 4.3) is similar to previous estimates (Esselstyn et al. 2009), differing only in the placement of *Crocidura mindorus*. The phylogenetic relationships of this species consistently receive low support (Esselstyn et al. 2009; Esselstyn and

Brown 2009; Esselstyn and Oliveros 2010), probably a result of rapid diversification (short internal branches) of Philippine species. However, as our test is based on branch lengths, the topology is only critical to the extent it affects branch lengths. The test statistic, ΔPatristic, was positive, and hence in the direction of overdispersion (Fig. 4.4); however, its deviation from zero was not statistically significant ($P = 0.272$).

Body-Size Dispersion

Body sizes, as indexed by skull length, range from 18.01 to 23.70 mm (Table 4.3). The empirical value of Δ*Size* (1.746) was greater than the corresponding values from nearly all randomizations (Fig. 4.5; $P = 0.012$), indicating that body size is significantly overdispersed in sympatric species pairs of shrews in the Philippines. Phylogenetic signal in body size was nearly significant ($P = 0.076$).

Island Colonization Process

Our simulations of island colonization suggest it is somewhat unlikely that shrews would colonize all of the currently occupied islands with the minimum necessary number of dispersal events. When the probability of colonization is inversely proportional to distance, the average number of colonization events necessary for shrews to reach 8 of 14 islands is 16.52, 8 of 9 islands (includes oceanic islands) is 32.25 and for shrews to colonize 5 of 6 islands (only the six largest Pleistocene islands included) it is 13.11 (Fig. 4.6). When we make long distance colonization more difficult by using the inverse of squared distances as the probability of colonization, the mean number of dispersal events increases dramatically to 56.89, 181.96 and 49.51, respectively (Fig. 4.6). The minimum number of colonization events necessary for *Crocidura* to reach all of the islands it is

known to occur on, with two species occurring sympatrically on three islands and one species on all other islands, is 10 (excluding colonization of the first island). This small number of colonization events was rare in the two simulation schemes that required colonization of 8 of 9 islands ($P \leq 0.017$; Fig. 4.6). In simulations with a termination criterion of 8 of 14 islands colonized, replicates with 10 or fewer colonization events were somewhat common when long distance colonization was probable ($P = 0.1404$), but rare when long-distance colonization was unlikely ($P = 0.0103$; Fig. 4.6). If we ignore shrew populations on oceanic islands, only considering the six PAICS (five of which are known to have shrew populations), the minimum necessary number of colonization events that can explain this distribution (three PAICs with two species, two PAICs with one species) is seven. Simulation replicates with seven or fewer colonization events were relatively common when the colonization probability was inversely proportional to distance ($P = 0.246$), but rare when long-distance colonization was simulated as more difficult ($P = 0.051$; Fig. 4.6).

Table 4.2. Results of background similarity tests of the predicted niches of *Crocidura beatus* and *C. grayi* using climate data for the present.

Similarity Metric	**Empirical Values**	***P*-values: *C. grayi* localities randomized**	***P*-values: *C. beatus* localities randomized**
Hoellinger's based I	0.9328	0.629	0.098
Schoener's D	0.9823	0.300	0.537

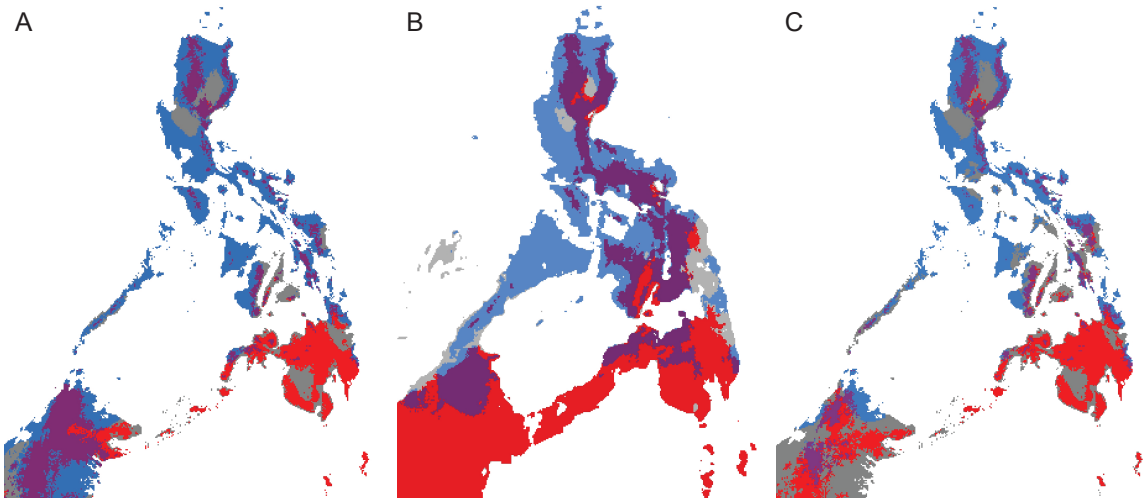


Figure 4.2. Results of ecological niche modeling, showing that potentially suitable climatic space for *Crocidura beatus* (red) and *C. grayi* (blue) in the Philippines and northern Borneo overlap broadly (purple). Areas identified by the niche models as unsuitable for both species are shown in gray. The predicted potential distributions during the Last Interglacial (A), Last Glacial Maximum (B), and present (C) are shown.

Table 4.3. Mean condylo-incisive lengths (mm), with standard errors and sample sizes for Philippine species of *Crocidura*, taken from voucher specimens collected on Pleistocene islands and oceanic islands. These lengths were used as a proxy for body size.

Species	**Island**	**Mean condylo-incisive length ± SE (*n*)**
C. batakorum	Palawan	18.01 ± 0.091 (5)
C. beatus	Greater Mindanao	20.99 ± 0.143 (13)
C. beatus	Camiguin	20.80 ± 0.136 (6)
C. grandis	Mindanao	23.70 ± NA (1)
C. grayi	Luzon	20.12 ± 0.091 (23)
C. grayi	Calayan	21.17 ± 0.170 (4)
C. grayi	Mindoro	19.63 ± 0.032 (15)
C. mindorus	Mindoro	22.28 ± 0.141 (4)
C. negrina	Negros	22.93 ± 0.215 (8)
C. palawanensis	Greater Palawan	23.62 ± 0.145 (27)
C. panayensis	Panay	21.45 ± 0.279 (7)
C. sp. nov.	Sibuyan	22.60 ± 0.335 (3)

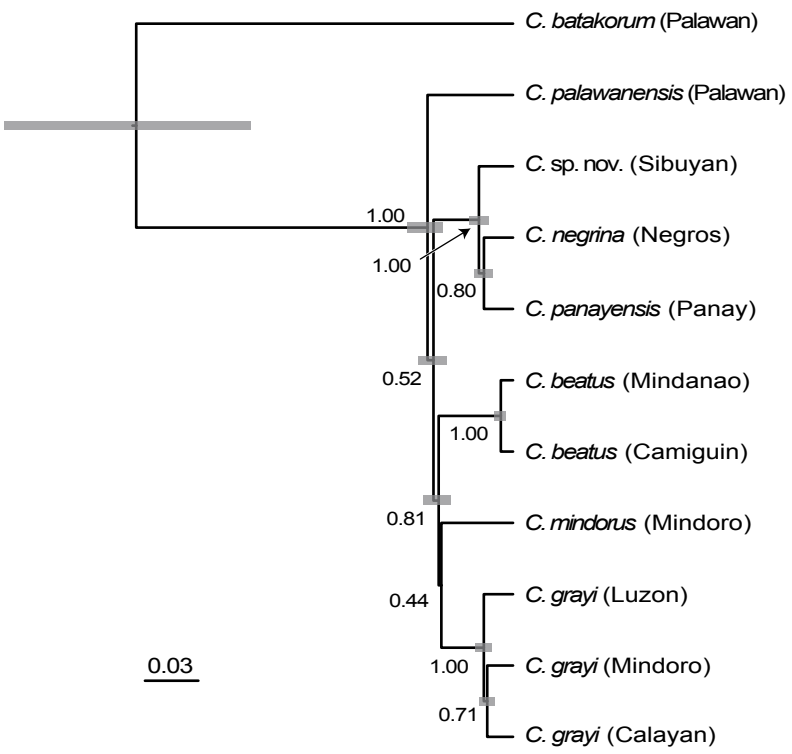


Figure 4.3. Maximum clade credibility tree for Philippine shrews (genus *Crocidura*) inferred using a relaxed log-normal clock with a mean substitution rate of 1.0. Terminals are labeled with species names, followed by island names in parentheses. Numbers at internal nodes are posterior probabilities. Gray bars at nodes represent 95% highest posterior densities of node ages on an arbitrary time scale.

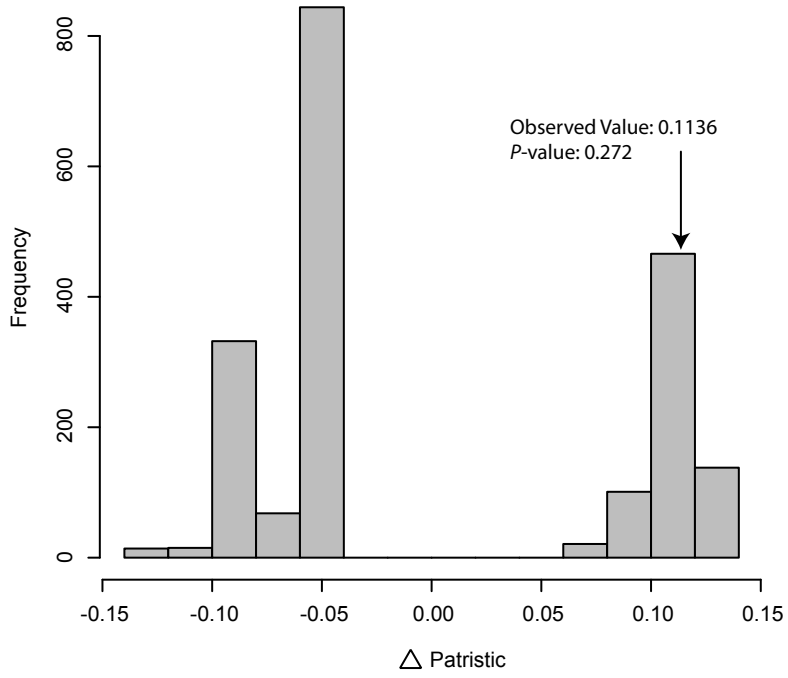


Figure 4.4. Distribution of 2000 randomizations of Δ*Patristic* (difference in mean patristic distances between sympatric species pairs and between allopatric pairs of species) among species of Philippine *Crocidura*. The observed value is indicated.

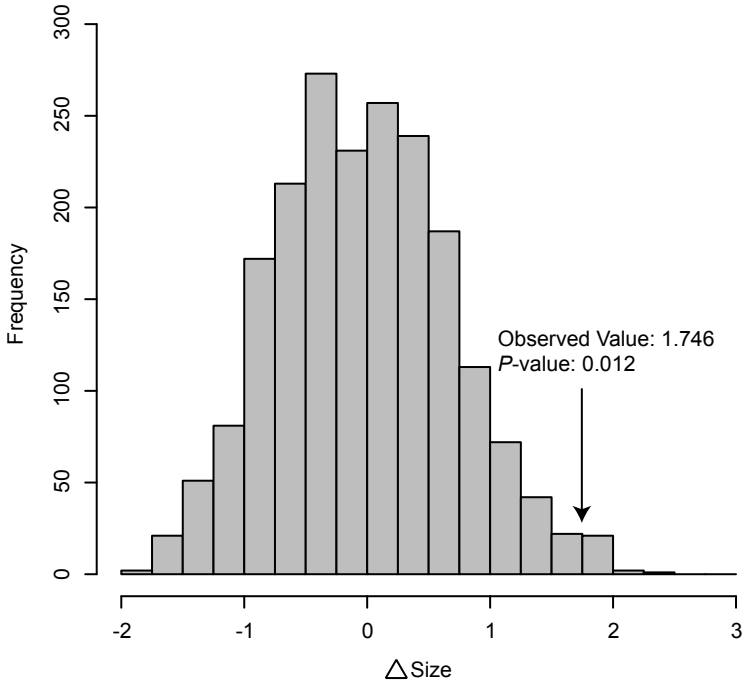
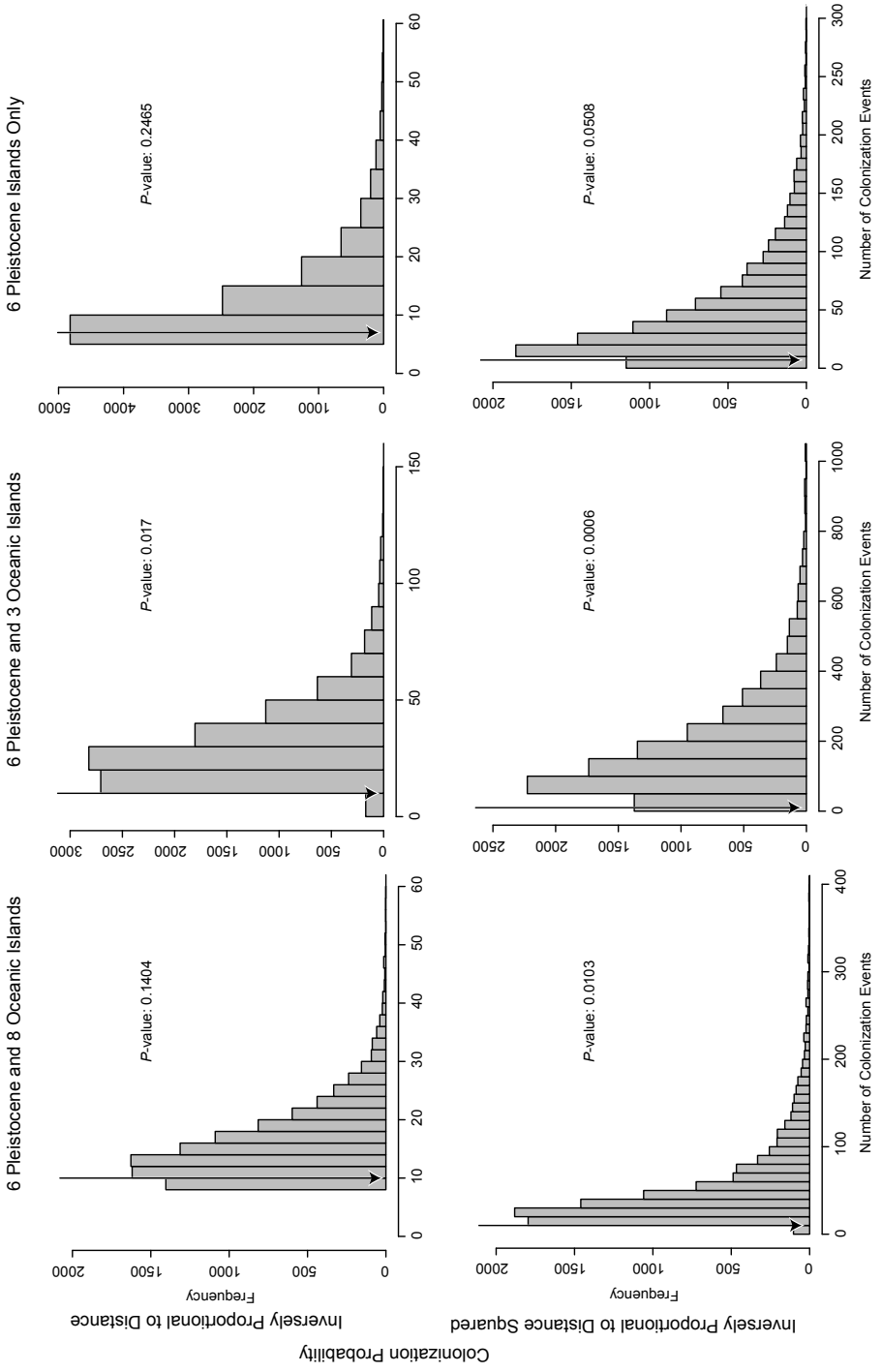


Figure 4.5. Distribution of 2000 randomizations of Δ*Size* (difference in the mean difference in skull length between sympatric species pairs and between allopatric pairs of species) among species of Philippine *Crocidura*. The observed value is indicated.

Figure 4.6. Histograms showing numbers of inter-island colonization events necessary to generate a geographic distribution similar to that of Philippine *Crocidura*. Vertical arrows indicate the minimum number of colonization events necessary to generate shrew populations on 8 of 14 islands, 8 of 9 islands, and 5 of 6 islands, in each case with three islands holding two species and all others holding one species. *P*-values indicate the proportion of simulations with the number of colonization events less than or equal to the minimum. Scales on x- and y-axes are not equal.


6 Pleistocene Islands Only
6 Pleistocene and 3 Oceanic Islands
6 Pleistocene and 8 Oceanic Islands
P-value: 0.2465
P-value: 0.0508
P-value: 0.017
P-value: 0.0006
P-value: 0.1404
P-value: 0.0103
Number of Colonization Events
Frequency
Inversely Proportional to Distance
Inversely Proportional to Distance Squared
Colonization Probability

Discussion

Each of our approaches provides weak evidence that competition may have played a role in determining current patterns of diversity of Philippine shrews. Our comparisons of ecological niche models for the two well-sampled species failed to reject similarity of ecological niches, leaving open the possibility for competitive interactions if the two species come into contact. Our tests of phylogenetic dispersion were in the direction of overdispersion (i.e., $\Delta Patristic > 0$), but not significant. However, some degree of overdispersion is expected in this situation—Esselstyn et al. (2009) used tests of alternative phylogenetic topologies to show that sympatric species of shrews in the Philippines are not sister species. Because all speciation events in this clade are the result of inter-island colonization, some overdispersion is expected. Statistical power for this test is almost certainly limited because only two pairs of sympatric species are included (i.e., no tissue samples are available for *C. grandis*). In contrast, our test of body-size dispersion, which included all three sympatric species pairs, demonstrates that co-occurring species are unexpectedly divergent in body size. Differences in body size among co-occurring species could be due to either character displacement or a body-size filter that prevents some species from colonizing occupied islands. We suspect the latter, as there appears to be some phylogenetic signal in body size.

Our simulations of the island colonization process indicate that under some scenarios (especially when long-distance colonization is difficult), it is unlikely that the islands that currently hold shrew populations could be colonized with the minimum necessary number of inter-island dispersal events. In other words, if competitive exclusion (or some other factor) is not preventing colonization (and resulting allopatric speciation), we expect to see a very different distribution of species on these islands. In

particular, there should be greater variation in species richness among islands. In contrast to this expectation, we see a highly regular pattern, in which all moderately large islands have only one or two species. However, we acknowledge that our decisions regarding which islands should be included in the colonization simulations directly affect these expectations. For instance, if we have excluded islands with shrew populations from the simulations, then our estimates of the numbers of colonization events necessary to populate the archipelago are too low, but if we have excluded islands that truly lack shrew populations, then our simulations would over estimate the numbers of colonization events.

In general, large PAICs have been the subject of more intensive biological surveys than have the smaller islands. Given this bias in survey effort, we decided to limit the simulations geographically to the largest PAICs, oceanic islands known to have shrew populations, and islands ≥ 100 km^2 and with records of at least three native mammal species. We thereby assumed the existence of a lower limit on the area of an island necessary to support a shrew population over long time scales. Among the islands in the area considered here that are known to have shrew populations, Maripipi is the smallest (22 km^2). However, it was united repeatedly with the larger islands of Greater Mindanao during the Pleistocene. Thus, the area of Maripipi may not provide a meaningful indication of the smallest island capable of sustaining a shrew population over long time scales. Calayan (196 km^2) is the smallest island never to have been connected to another island that is known to have a population of *Crocidura*. However, outside the area of our geographic focus, populations of *Crocidura* are found on Batan (35 km^2) and Sabtang (41 km^2) islands (Esselstyn and Oliveros 2010; Heaney and Ruedi 1994), which were connected to each other by low sea levels during Pleistocene glacial

cycles, but are isolated from other islands and the continent by deep water. Thus, there is little evidence of shrews being capable of long-term persistence on islands smaller than about 100 km^2. Because the distribution of *Crocidura* in the Philippines is almost certainly incompletely known, we decided to adopt three geographic scopes in our simulations. In the first, we included all six PAICs (five of which have shrew populations) and eight oceanic islands (three with shrew populations). However, we note that the mammal faunas of the five oceanic islands lacking shrew records are very poorly known (Heaney et al. 2010), and it remains possible that shrew populations exist on at least some of those islands. In the second approach, we included all oceanic islands and all Pleistocene islands with a record of shrews, plus the largest PAIC that lacks a record (Sulu; Fig. 4.1), with the expectation that all but one of these islands be colonized. This scenario is liberal in that it excludes oceanic islands that probably have not been colonized, resulting in an increase in the number of colonization events necessary for simulated shrews to reach eight of nine islands. However, it is conservative (as are the other approaches) in that we treated PAICs as cohesive units that only need to be colonized once, despite evidence to the contrary. For instance, the populations of *C. beatus* on Samar and Leyte islands are deeply divergent from other populations on Greater Mindanao (Esselstyn and Brown 2009; Esselstyn et al. 2009). Our niche models indicate an area of unsuitable habitat between Leyte/Samar and Mindanao during the Last Glacial Maximum, when the islands were last united (Fig. 4.2B). This suggests that establishing a shrew population on these islands required an additional colonization event, as if it were an oceanic island. If modern islands within Pleistocene islands have been colonized over water, or over unsuitable habitats, then our decisions regarding geographic scope would cause us to underestimate numbers of colonization events

necessary to generate the known distribution of shrew species. Our hope is that these contrary potential biases balance one another out; however, because of the impossibility of knowing an island lacks shrews, it is difficult to decide which, if any of the adopted scopes, are reasonable. In our final approach, we ignored the existence of oceanic islands, only considering the six largest PAICs, five of which are known to have shrew populations. By excluding oceanic islands, we hope to bypass most of the uncertainty associated with the distribution of shrews. However, we note that even within PAICs, there is uncertainty. However, the lack of a record of shrews from Sulu may reflect the very limited efforts that have been expended to survey small mammals on these islands. Had we required colonization of all six islands (including Sulu), this would have greatly increased the numbers of colonization events.

If our chosen geographic scopes and colonization probabilities are reasonable, then numerous potential colonization events may have failed after dispersing shrews arrived on islands already occupied by another species. In effect, this constraint would have limited the number of speciation events by preventing the establishment of allopatric populations of individual species. This interpretation assumes that dispersing individuals would not simply interbreed with local populations. Unfortunately, we have no means of assessing whether these species have the capacity to interbreed. If dispersing individuals do interbreed with resident populations, then a genetic signal should be detectable in the form of polyphyly of island populations. However, the foreign genotypes might be extremely rare and detecting them would probably require extraordinarily dense sampling. The population-level samples used from *C. beatus* and *C. grayi* in a previous analysis (Esselstyn and Brown 2009) showed no signs of introgression, implying that dispersing individuals do not breed with resident populations.

However, we doubt that this sampling was sufficient to provide evidence against extremely rare inter-species introgression.

We further note that in two of the three cases of co-occurring *Crocidura*, one member of the sympatric pair is a restricted range species, perhaps endemic to a single mountain. Specifically, on Mindoro Island, *C. mindorus* is only known from near the peak of Mt. Halcon, but *C. grayi* is widespread and common on the island. Both species have been collected at high elevation on Mt. Halcon, suggesting they may be truly sympatric on that mountain. Similarly, on Mindanao Island, *C. grandis* is only known from the type locality at high elevation on Mt. Malindang, but *C. beatus* is widespread on the island and known from numerous localities, including areas sampled on Mt. Malindang. In both cases, surveys of neighboring mountains have failed to capture the apparent micro-endemic species (Esselstyn and Brown 2009; Esselstyn and Oliveros 2010; Heaney et al. 2006; Esselstyn and Goodman, in review; Esselstyn, D. S. Balete, L. R. Heaney unpubl. data). Thus, it appears that *C. mindorus* and *C. grandis* are each restricted to high elevation areas on one mountain, implying that a narrowing of one species' niche may facilitate coexistence. In contrast, on Palawan Island, *C. batakorum* and *C. palawanensis* are both widely distributed, and occur in true sympatry, at least at one site (Esselstyn et al. 2009). The patristic distances and differences in body size between these two species are greater than those observed in the other pairs of sympatric species, though it should be noted that *C. batakorum* is not a member of the same clade as the other Philippine species (Esselstyn et al. 2009).

Esselstyn et al. (2009) examined the tempo of speciation in Southeast Asian *Crocidura* on a broader scale than used here, and found that the rate of diversification has been relatively consistent, without a marked temporal decline in the speciation rate, as is

frequently noted in other groups (McPeek 2008; Phillimore and Price 2008). They concluded that the Southeast Asian *Crocidura* clade is either too young to have yet saturated its environment, or that ecological opportunity is not a limiting factor in geographically dynamic archipelagos. Results obtained here weakly suggest a role for ecological constraints, implying that the lack of decline in the net diversification rate across the region is simply an artifact of the clade's young age. However, it is important to recognize that the temporal and spatial scales at which potential drivers and constraints of diversification are examined may be crucial. For instance, it seems that very different evolutionary and ecological processes have generated diversity in different places, with allopatric speciation and niche conservatism the suggested pattern among Philippine shrews. Sulawesian *Crocidura*, in contrast, are likely the product of a very different set of processes (Esselstyn et al. 2009; Ruedi et al. 1998), in which sympatric species are each other's closest relatives, and ecological diversification has been a more important component of their evolutionary history.

Our results suggest Philippine shrews represent a primarily non-adaptive radiation, in which a lack of ecological innovation may have prevented the accumulation of more than two species per island. Although no explicit tests have been conducted, several other Philippine endemic clades appear to have diversified in a similar manner. For example, Philippine bulbuls appear to follow the non-adaptive path (Oliveros and Moyle 2010), as do fanged frogs (Evans et al. 2003a). Philippine murid rodents differ from these cases in that they possess a wide range of ecologies, and are more typical of an adaptive radiation (Heaney and Rickart 1990; Jansa et al. 2006). Although the distinction between adaptive and non-adaptive radiations is one of degree (Olson and Arroyo-Santos 2009), we suspect that most terrestrial vertebrates that have diversified

within the Philippines are closer to the non-adaptive end of the spectrum. If our supposition is correct, then a general lack of ecological innovation may present a greater hindrance to speciation than does the need to cross the numerous ocean channels that 'isolate' the many islands of the Philippines. Although our results are not conclusive, they provide a new perspective and set of testable hypotheses that potentially explain the accumulation of insular diversity, in which inter-island dispersal is common, but successful colonization rare, and a general lack of ecological innovation constrains archipelago-wide diversity.

Summary

Shrews have successfully invaded much of Southeast Asia, including numerous oceanic islands. Their presence on isolated islands such as Batan in the northern Philippines (Esselstyn and Oliveros 2010) and Aru on the Australian continental shelf (Kitchener et al. 1994) is a testament to their ability to disperse over oceanic barriers. Phylogenetic relationships are now much better understood for most Philippine species, but much remains unknown as to the diversity and endemism of *Crocidura* in the Indonesian archipelago and in Indochina (Jenkins et al. 2009; Kithchener et al. 1994; Ruedi 1995).

The Philippines has been invaded at least three times by *Crocidura*, including one colonization event from the north and two from the south. However, only one of these invasions produced *in situ* speciation. Sulawesi has been colonized at least twice, with one colonization event resulting in the production of perhaps eight species (Esselstyn et al. 2009). Shrew diversity on the Sunda Shelf is represented by multiple lineages, but because of the complex pattern of historical connection and isolation between the shelf and the Asian mainland, along with limited sampling from the area, it is difficult to speculate on the number and direction of colonization events.

Most species-level diversity in Southeast Asian *Crocidura* has probably been generated via allopatric speciation following over-water colonization events. In the Philippines, most species are allopatric and appear to have only minor differences in ecologically important traits. In the few cases of sympatry among Philippine species of *Crocidura*, differences in body size are statistically significant and anecdotal evidence suggests the narrowing of ecological niches (i.e., specialization on high-elevation habitats) in one member of the sympatric pairs may facilitate coexistence. The potential presence of a sympatry threshold and an apparent lack of ecological diversificaton (at

least between the well sampled *C. beatus* and *C. grayi*) may have limited diversity in the Philippines by causing the failure of inter-island dispersal events to establish new, isolated populations. However, in contrast, shrew diversity on Sulawesi may be the result of sympatric speciation and ecological diversification. At least five closely related species co-occur at a single site in the central part of the island (Ruedi 1995), and these appear to be more diverse ecologically than the entire assemblage of mostly allopatric Philippine species. Unfortunately, the number of specimens available from Sulawesi is severely limited and prevents an explicit test of this hypothesis.

At a broader scale, the entirety of Southeast Asian shrews appears to have diversified at a fairly consistent tempo. There is only weak evidence for a very gradual decline in the speciation rate through time (Esselstyn et al. 2009). No significant shifts in diversification rate were found, and thus there is no evidence of a link between speciation rates and geological or climatic processes such as Pleistocene sea-level fluctuations. The steady tempo of diversification suggests that this clade is either too young to have yet filled available ecological space, or that the dynamic nature of the archipelago has provided sufficient new opportunities for isolation as to enable continual diversification across geography. The very recent colonization of Batan and Sabtang islands (and perhaps Aru) suggests that Southeast Asian shrews represent an immature radiation that is still expanding its geographic range. My suspicion is that this clade is too young to have yet saturated its environment and that ecological similarity among allopatric species on neighboring islands has prevented the accumulation of diversity in some areas, but has not been an important factor in other areas. Careful examination of the temporal and spatial scales at which these processes alter diversification trajectories may provide additional insights.

Literature Cited

Abramov, A. V., P. D. Jenkins, V. V. Rozhnov, and A. A. Kalinin. 2008. Description of a new species of *Crocidura* (Soricomorpha: Soricidae) from the island of Phu Quoc, Vietnam. Mammalia 72:269–272.

Allen, D., C. Espanola, G. Broad, C. Oliveros, and J. C. T. Gonzalez. 2006. New bird records from the Babuyan islands, Philippines, including two first records for the Philippines. Forktail 22:57–70.

Anderson, R. P., A. T. Peterson, and M. Gómez-Laverde. 2002. Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. Oikos 98:3–16.

Arbogast, B. S., S. V. Edwards, J. Wakeley, P. Beerli, and J. B. Slowinski. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annual Review of Ecology and Systematics 33:707–740.

Bannikova, A. A., V. S. Lebedev, D. A. Kramerov, M. V. Zaitsev. 2006. Phylogeny and systematics of the *Crocidura suaveolens* species group: corroboration and controversy between nuclear and mitochondrial DNA markers. Mammalia 70:106–119.

Beyer, H. L. 2004. Hawth's analysis tools for ArcGIS. Available at http://www.spatialecology.com/htools.

Bintanja, R., R. S. W. van de Wal, and J. Oerlemans. 2005. Modeled atmospheric temperatures and global sea levels over the past million years. Nature 437:125–128.

Bird, M. I., D. Taylor, and C. Hunt. 2005. Palaeoenvironments of insular Southeast Asia during the Last Glacial Period: a savanna corridor in Sundaland? Quaternary Science Review 24:2228–2242.

Brandli, L., L. Handley, P. Vogel, N. Perrin. 2005. Evolutionary history of the greater white-toothed shrew (*Crocidura russula*) inferred from analysis of mtDNA, Y, and X chromosome markers. Molecular Phylogenetics and Evolution 37:832–844.

Brown, R. M., and A. C. Diesmos. 2002. Application of lineage-based species concepts to oceanic island frog populations: the effects of differing taxonomic philosophies on the estimation of Philippine biodiversity. Silliman Journal 42:133–162.

Brown, R. M., and A. C. Diesmos. 2009. The Philippines, Biology. Pages 723–732 *in* R. Gillespie and D. Clague, eds. Encyclopedia of Islands. University of California Press, Berkeley.

Brown, R. M., and S. I. Guttman. 2002. Phylogenetic systematics of the *Rana signata* complex of Philippine and Bornean stream frogs: reconsideration of Huxley's modification of Wallace's Line at the Oriental–Australian faunal zone interface. Biological Journal of the Linnean Society 76:393–461.

Brown, R. M., C. H. Oliveros, C. D. Siler, A. C. Diesmos. 2009a. Phylogeny of *Gekko* from the northern Philippines, and description of a new species from Calayan Island. Journal of Herpetology 43:620–635.

Brown, R. M., C. D. Siler, A. C. Diesmos, and A. C. Alcala. 2009b. The Philippine frogs of the genus *Leptobrachium* (Anura; Megophryidae): phylogeny-based species delimitation, taxonomic revision, and descriptions of three new species. Herpetological Monographs 23:1–44.

Butler, P. M. 1998. Fossil history of shrews in Africa. Pages 121–132 *in* J. M. Wójcik and M. Wolson, eds. Evolution of shrews. Mammal Research Institute, Polish Academy of Sciences, Białowieża.

Carranza, S., E. N. Arnold, J. A. Mateo, and L. F. López-Jurado. 2002. Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. Proceedings of the Royal Society B: Biological Sciences 267:637–649.

Carstens, B. C., A. L. Stevenson, J. D. Degenhardt, and J. Sullivan. 2004. Testing nested phylogenetic and phylogeographic hypotheses in the *Plethodon vandykei* species group. Systematic Biology 53:781–792.

Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. The American Naturalist 163:823–843.

Chan, R. C. Y., G. Reid, R. T. Irvin, A. W. Bruce, and J. W. Costerton. 1985. Competitive exclusion of uropathogens from human uroepithelial cells by *Lactobacillus* whole cells and cell wall fragments. Infection and Immunity 47:84–89.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

Connell, J. H. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35:131–138.

Cooper, N., J. Rodríguez, and A. Purvis. 2008. A common tendency for phylogenetic overdispersion in mammalian assemblages. Proceedings of the Royal Society of London, B, Biological Sciences 275:2031–2037.

Corbet, G. B., and J. E. Hill. 1992. The mammals of the Indomalayan region. Oxford University Press, New York.

Cowie, R. H., and B. S. Holland. 2006. Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. Journal of Biogeography 33:193–198.

Darwin, C. 1859. The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. John Murray, London.

Defant, M. J., R. C. Maury, J.-L. Joron, M. D. Feigenson, J. Leterrier, H. Bellon, D. Jacques, and M. Richard. 1990. The geochemistry and tectonic setting of the northern section of the Luzon arc (the Philippines and Taiwan). Tectonophysics 183:187–205.

Delacour, J., and E. Mayr. 1946. Birds of the Philippines. MacMillan, New York.

Den Boer, P. J. 1986. The present status of the competitive exclusion principle. Trends in Ecology and Evolution 1:25–28.

de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. Pages 55–75 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford University Press, Oxford.

Diamond, J. 1986. Evolution of ecological segregation in the New Guinea montane avifauna. Pages 98–125 *in* J. Diamond and T. J. Case, eds. Community Ecology. Harper & Row, New York.

Diamond, J., S. L. Pimm, M. E. Gilpin, and M. LeCroy. 1989. Rapid evolution of character displacement in myzomelid honeyeaters. The American Naturalist 134:675–708.

Diamond, J. M. 1975. Assembly of species communities. Pages 342–344 *in* M. L. Cody and J. M. Diamond, eds. Ecology and evolution of communities. Belknap, Cambridge, MA.

Diamond, J. M., and M. E. Gilpin. 1983. Biogeographic umbilici and the origin of the Philippine avifauna. Oikos 41:307–321.

Dib, L., I. Bitam, M. Tahri, M. Bensouilah, and T. De Meeûs. 2008. Competitive exclusion between piroplasmosis and anaplasmosis agents within cattle. PLoS Pathogens 4:e7. doi:101371/journal.ppat.0040007.

Dickerson, R. E. 1928. Distribution of life in the Philippines. Philippine Bureau of Science, Manila.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia University Press, New York.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214.

Drummond, A. J., A. Rambaut, B. Shapiro, O. G. Pybus. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22:1185–1192.

Dubey, S., P. Nová, P. Vogel, V. Vohralík. 2007a. Cytogenetic and molecular relationships between Zarudny's rock shrew (*Crocidura zarudnyi*; Mammalia: Soricomorpha) and Eurasian taxa. Journal of Mammalogy 88:706–711.

Dubey, S., N. Salamin, S. D. Ohdachi, P. Barriere, and P. Vogel. 2007b. Molecular phylogenetics of shrews (Mammalia: Soricidae) reveal timing of transcontinental colonizations. Molecular Phylogenetics and Evolution 44:126–137.

Dubey, S., N. Salamin, M. Ruedi, P. Barriere, M. Colyn, and P. Vogel. 2008. Biogeographic origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution 48:953–963.

Díaz-Pérez, A., M. Sequeira, A. Santos-Guerra, and P. Catalán. 2008. Multiple colonization, in situ speciation, and volcanism-associated stepping-stone dispersals shaped the phylogeography of the Macaronesian red fescues (*Festuca* L., Gramineae). Systematic Biology 57:732–749.

Ellerman, J. R., and T. C. S. Morrison-Scott. 1951. Checklist of Palaearctic and Indian mammals, 1758 to 1946. British Museum (Natural History), London.

Emerson, B. C. 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11:951–966.

Esselstyn, J. A., and R. M. Brown. 2009. The role of repeated sea level fluctuations in the generation of shrew diversity in the Philippine Archipelago. Molecular Phylogenetics and Evolution 53:171–181.

Esselstyn, J. A., H. J. D. Garcia, M. G. Saulog, and L. R. Heaney. 2008. A new species of *Desmalopex* (Pteropodidae) from the Philippines, with a phylogenetic analysis of the Pteropodini. Journal of Mammalogy 89:215–225.

Esselstyn, J. A., and S. M. Goodman. In review. A new species of shrew (Soricidae: *Crocidura*) from Sibuyan Island, Philippines. Journal of Mammalogy.

Esselstyn, J. A., and C. H. Oliveros. 2010. Colonization of the Philippines from Taiwan: a multilocus test of the biogeographic and phylogenetic relationships of isolated populations of shrews. Journal of Biogeography. doi:10.1111/j.1365-2699.2010.02295.x.

Esselstyn, J. A., C. H. Oliveros, R. G. Moyle, A. T. Peterson, J. A. McGuire, and R. M. Brown. In review. Integrating phylogenetic and taxonomic evidence illuminates complex biogeographic patterns along Huxley's modification of Wallace's Line. Journal of Biogeography.

Esselstyn, J. A., R. M. Timm, and R. M. Brown. 2009. Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. Evolution 63:2595–2610.

Esselstyn, J. A., P. Widmann, and L. R. Heaney. 2004. The mammals of Palawan Island, Philippines. Proceedings of the Biological Society Washington 117:271–302.

Estoup, A., and S. M. Clegg. 2003. Bayesian inferences on the recent island colonization history by the bird Zosterops lateralis lateralis. Molecular Ecology 12:657–674.

Evans, B. J., J. A. McGuire, R. M. Brown, N. Andayani, and J. Supriatna. 2008. A coalescent framework for comparing alternative models of population structure with genetic data: evolution of Celebes toads. Biology Letters 4:430–433.

Evans, B. J., J. C. Morales, J. Supriatna, and D. J. Melnick. 1999. Origin of the Sulawesi macaques (Cercopithecidae: *Macaca*) as suggested by mitochondrial DNA phylogeny. Biological Journal of the Linnean Society 66:539–560.

Evans, B. J., R. M. Brown, J. A. McGuire, J. Supriatna, N. Andayani, A. Diesmos, D. Iskandar, D. J. Melnick, and D. C. Cannatella. 2003a. Phylogenetics of fanged frogs: testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. Systematic Biology 52:794–819.

Evans, B. J., J. Supriatna, N. Andayani, M. I. Setiadi, D. C. Cannatella, and D. J. Melnick. 2003b. Monkeys and toads define areas of endemism on Sulawesi. Evolution 57:1436–1443.

Everett, A. H. 1889. Remarks on the zoogeographical relationships of the island of Palawan and some adjacent islands. Proceedings of the Zoological Society of London 1889:220–228.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Fang, Y. P., and L. L. Lee. 2002. Re-evaluation of the Taiwanese white-toothed shrew, Crocidura tadae Tokuda and Kano, 1936 (Insectivora: Soricidae) from Taiwan and two offshore islands. Journal of Zoology 257:145–154.

Fang, Y. P., L. L. Lee, F. H. Yew, and H. T. Yu. 1997. Systematics of white-toothed shrews (*Crocidura*) (Mammalia: Insectivora: Soricidae) of Taiwan: karyological and morphological studies. Journal of Zoology 242:151–166.

Felsenstein, J. 2004. Inferring phylogenies. Sinauer Associates, Inc., Sunderland.

Filardi, C. E., and R. G. Moyle. 2005. Single origin of a pan-Pacific bird group and upstream colonization of Australasia. Nature 438:216–219.

Fumagalli, L., Taberlet, P., Stewart, D.T., Gielly, L., Hausser, J., Vogel, P., 1999. Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 11:222–235.

Gause, G. F. 1934. The struggle for existence. Williams & Wilkins Co., Baltimore.
Gillespie, R. G., H. B. Croom, and S. R. Palumbi. 1994. Multiple origins of a spider radiation in Hawaii. Proceedings of the National Academy of Sciences USA 91:2290–2294.
Gissi, C., A. Reyes, G. Pesole, C. Saccone. 2000. Lineage-specific evolutionary rate in mammalian mtDNA. Molecular Biology and Evolution 17:1022–1031.
Gorog, A. J., M. H. Sinaga, and M. D. Engstrom. 2004. Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). Biological Journal of the Linnean Society 81:91–109.
Gould, S. J., and R. C. Lewontin. 1979. Spandrels of San Marco and the Panglossian paradigm – A critique of the adaptationist program. Proceedings of the Royal Society, B. 205:581–598.
Grant, P. R. 1986. Interspecific competition in fluctuating environments. Pages 173–191 *in* J. Diamond and T. J. Case, eds. Community Ecology. Harper & Row, New York.
Grant, P. R., B. R. Grant, and K. Petren. 2000. The allopatric phase of speciation: the sharp-beaked ground finch (*Geospiza difficilis*) on the Galapagos islands. Biological Journal of the Linnean Society 69:287–317.
Gray, R. D., A. J. Drummond, and S. J. Greenhill. 2009. Language phylogenies reveal expansion pulses and pauses in Pacific settlement. Science 323:479–483.
Grinnell, J. 1904. The origin and distribution of the chest-nut-backed chickadee. Auk 21:364–382.
Gurevitch, J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. The American Naturalist 140:539–572.
Hall, R. 1998. The plate tectonics of Cenozoic Southeast Asia and the distribution of land and sea. Pages 99–131 *in* R. Hall and J. D. Holloway, eds. Biogeography and geological evolution of Southeast Asia. Backhuys Publishers, Leiden.
Hall, R. 2002. Cenozoic geological and plate tectonic evolution of Southeast Asia and the SW Pacific: computer-based reconstructions and animations. Journal of Asian Earth Sciences 20:353–434.
Haq, B. U., J. Hardenbol, and P. R. Vail. 1987. Chronology of fluctuating sea levels since the Triassic. Science 235:1156–1167.
Hardin, G. 1960. The competitive exclusion principle. Science 131:1292–1297.
Harmon, L. J., J. Melville, A. Larson, and J. B. Losos. 2008. The role of geography and ecological opportunity in the diversification of day geckos (*Phelsuma*). Systematic Biology 57:562–573.
Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.
Heaney, L. R. 1984. Mammalian species richness on islands on the Sunda Shelf, Southeast Asia. Oecologia 61:11–17.
Heaney, L. R. 1985. Zoogeographic evidence for middle and late Pleistocene land bridges to the Philippine Islands. Modern Quaternary Research in Southeast Asia 9:127–144.
Heaney, L. R. 1986. Biogeography of mammals in Southeast Asia: estimates of rates of colonization, extinction and speciation. Biological Journal of the Linnean Society 28:127–165.

Heaney, L. R. 1991. A synopsis of climatic and vegetational change in Southeast Asia. Climate Change 19:53–61.

Heaney, L. R. 2000. Dynamic disequilibrium: a long-term, large-scale perspective on the equilibrium model of island biogeography. Journal of Biogeography 9:59–74.

Heaney, L. R. 2007. Is a new paradigm emerging for oceanic island biogeography? Journal of Biogeography 34:753–757.

Heaney, L. R., D. S. Balete, M. L. Dolar, A. C. Alcala, A. T. L. Dans, P. C. Gonzales, N. R. Ingle, M. V. Lepiten, W. L. R. Oliver, P. S. Ong, E. A. Rickart, B. R. Tabaranza, Jr., and R. B. Utzurrum. 1998. A synopsis of the mammalian fauna of the Philippine Islands. Fieldiana: Zoology n.s. 88:1–61.

Heaney, L. R., M. L. Dolar, D. S. Balete, J. A. Esselstyn, E. A. Rickart, and J. L. Sedlock. 2010. Synopsis of Philippine mammals. http://www.fieldmuseum.org/philippine_mammals/.

Heaney, L. R., and E. A. Rickart. 1990. Correlations of clades and clines: Geographic, elevational, and phylogenetic distribution patterns among Philippine mammals. Pages 321–332 *in* G. Peters and R. Hutterer, eds. Vertebrates in the Tropics. Alexander Koenig Zoological Research Institute and Zoological Museum, Bonn.

Heaney, L. R., and M. Ruedi. 1994. A preliminary analysis of biogeography and phylogeny of *Crocidura* from the Philippines. Special Publication of the Carnegie Museum of Natural History 18:357–377.

Heaney, L. R., and B. R. Tabaranza, Jr. 2006. Mammal and land bird studies on Camiguin Island, Philippines: Background and conservation priorities. Fieldiana: Zoology n.s. 106:1–13.

Heaney, L. R., B. R. Tabaranza, Jr., E. A. Rickart, D. S. Balete, and N. R. Ingle. 2006. The mammals of Mt. Kitanglad Nature Park, Mindanao Island, Philippines. Fieldiana: Zoology n.s. 112:1–63.

Heaney, L. R., E. K. Walker, B. R. Tabaranza, Jr., and N. R. Ingle. 2002. Mammalian diversity in the Philippines: an assessment of the adequacy of current data. Sylvatrop: Technical Journal of Philippine Ecosystems and Natural Resources 10:6–27.

Heaney, L. R., J. S. Walsh, Jr., and A. T. Peterson. 2005. The roles of geological history and colonization abilities in genetic differentiation between mammalian populations in the Philippine archipelago. Journal of Biogeography 32:229–247.

Hijmans R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

Hutchinson, G. E. 1957. Homage to Santa Rosalia or why are there so many kinds of animals. The American Naturalist 93:145–159.

Hutterer, R. 2005. Order Soricomorpha. Pages 220–311 *in* D. E. Wilson and D. M. Reeder, eds. Mammal species of the world. Johns Hopkins University Press, Baltimore.

Hutterer, R. 2007. Records of shrews from Panay and Palawan, Philippines, with the description of two new species of *Crocidura* (Mammalia: Soricidae). Lynx 38:5–20.

Inger, R. F. 1954. Systematics and zoogeography of Philippine Amphibia. Fieldiana: Zoology 33:181–531.

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome-B gene of mammals. Journal of Molecular Evolution 32:128–144.

Jansa, S. A., F. K. Barker, and L. R. Heaney. 2006. The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. Systematic Biology 55:73–88.

Jenkins, P. D., A. A. Abramov, V. V. Rozhnov, O. V. Makarova. 2007. Description of two new species of white-toothed shrews belonging to the genus *Crocidura* (Soricomorpha: Soricidae) from Ngoc Linh Mountain, Vietnam. Zootaxa 1589:57–68.

Jenkins, P. D., D. P. Lunde, C. B. Moncrieff. 2009. Descriptions of new species of *Crocidura* (Soricomorpha: Soricidae) from mainland Southeast Asia, with synopses of previously described species and remarks on biogeography. Bulletin of the American Museum of Natural History 331:356–405.

Jiménez-Valverde, A., Y. Nakazawa, A. Lira-Noriega, and A. T. Peterson. 2009. Environmental correlation structure and ecological niche model projections. Biodiversity Informatics 6:28–35.

Jones, A. W., and R. S. Kennedy. 2008. Evolution in a tropical archipelago: comparative phylogeography of Philippine fauna and flora reveals complex patterns of colonization and diversification. Biological Journal of the Linnean Society 95:620–639.

Jordan, D. S. 1908. The law of geminate species. The American Naturalist 42:73–80.

Kirkland, G. L. 1991. Competition and co-existence in shrews. Pages 15–22 *in* J. S. Findley and T. L. Yates, eds. The Biology of the Soricidae. Special Publication of the Museum of Southwestern Biology, Albuquerque.

Kitchener, D. J., S. Hisheh, L. H. Schmitt, and A. Suyanto. 1994. Shrews (Soricidae: Crocidura) from the Lesser Sunda Islands, and southeast Maluku, eastern Indonesia. Australian Mammalogy 17:7–17.

Klein, N. K., and W. M. Brown. 1994. Intraspecific molecular phylogeny in the yellow warbler (*Dendroica petechia*), and implications for avian biogeography in the West Indies. Evolution 48:1914–1932.

Kloss, B. 1929. The zoo-geographical boundaries between Asia and Australia and some Oriental sub-regions. Bulletin of the Raffles Museum 2:1–10.

Knowles, L. L., and B. C. Carstens. 2007. Delimiting species without monophyletic gene trees. Systematic Biology 56:887–895.

Kozak, K. H., D. W. Weisrock, and A. Larson. 2006. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: *Plethodon*). Proceedings of the Royal Society of London, Series B 273:539–546.

Lack, D. 1947. Darwin's Finches. University Press, Cambridge.

Lunde, D. P., G. G. Musser, and T. Ziegler. 2004. Description of a new species of *Crocidura* (Soricomorpha: Sorcidae: Crocidurinae) from Ke Go Nature Reserve, Vietnam. Mammal Study 29:27–36.

MacArthur, R. H. 1972. Geographical ecology. Harper & Row, New York.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Marshall, D. C., K. Slon, J. R. Cooley, K. B. R. Hill, and C. Simon. 2008. Steady Plio–Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation. Molecular Phylogenetics and Evolution 48:1054–1066.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

Mayr, E. 1963. Animal species and evolution. Belknap Press, Cambridge, MA.

McGuire, J. A., and B. H. Kiew. 2001. Phylogenetic systematics of Southeast Asian flying lizards (Iguania: Agamidae: *Draco*) as inferred from mitochondrial DNA sequence data. Biological Journal of the Linnean Society 72:203–229.

McPeek, M. A. 2008. The ecological dynamics of clade diversification and community assembly. The American Naturalist 172:E270–284.

Meijaard, E., and A. P. M. van der Zon. 2003. Mammals of Southeast Asian islands and their late Pleistocene environments. Journal of Biogeography 30:1245–1257.

Miller, G. S. 1910. Descriptions of two new genera and sixteen new species of mammals from the Philippine Islands. Proceedings of the U.S. National Museum 38:391–404.

Miller, K. G., M. A. Kominz, J. V. Browning, J. D. Wright, G. S. Mountain, M. E. Katz, P. J. Sugarman, B. S. Cramer, N. Christie-Blick, and S. F. Pekar. 2005. The Phanerozoic record of global sea-level change. Science 310:1293–1298.

Mittermeier, R. A., P. R. Gil, M. Hoffmann, J. Pilgrim, T. Brooks, C. G. Mittermeier, J. Lamoreux, and G. A. B. Da Fonesca. 2004. Hotspots Revisited. CEMEX, Mexico City.

Moen, D. S., and J. J. Wiens. 2009. Phylogenetic evidence for competitively driven divergence: body-size evolution in Caribbean treefrogs (Hylidae: *Osteopilus*). Evolution 63:195–214.

Motokawa, M., H. T. Yu, and M. Harada. 2005. Diversification of the white-toothed shrews of the genus *Crocidura* (Insectivora: Soricidae) in East and Southeast Asia. Mammal Study 30:S53–S64.

Nee, S. 2001. Inferring speciation rates from phylogenies. Evolution 55:661–668.

Neill, C., T. Daufresne, and C. G. Jones. 2009. A competitive coexistence principle? Oikos 118:1570–1578.

Nevo, E., 1978. Genetic variation in natural populations: patterns and theory. Theoretical Population Biology 13:121–177.

Nicholson, K. E., R. E. Glor, J. J. Kolbe, A. Larson, S. B. Hedges, J. B. Losos. 2005. Mainland colonization by island lizards. Journal of Biogeography 32:929–938.

Nylander, J. A. A., J. C. Wilgenbusch, D. L. Warren, and D. L. Swofford. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581–583.

Ohdachi, S. D., M. Hasegawa, M.-A. Iwasa, P. Vogel, T. Oshida, L.-K. Lin, and H. Abe. 2006. Molecular phylogenetics of soricid shrews (Mammalia) based on mitochondrial cytochrome b gene sequences: with special reference to the Soricinae. Journal of Zoology 270:177–191.

Ohdachi, S. D., M.-A. Iwasa, V. A. Nesterenko, H. Abe, R. Masuda, W. Haberl. 2004. Molecular phylogenetics of *Crocidura* shrews (Insectivora) in east and central Asia. Journal of Mammalogy 85:396–403.

Oliveros, C. H., and R. G. Moyle. 2010. Origin and diversification of Philippine bulbuls. Molecular Phylogenetics and Evolution 54:822–832.

Olson, L. E., S. M. Goodman, and A. D. Yoder. 2004. Illumination of cryptic species boundaries in long-tailed shrew tenrecs (Mammalia: Tenrecidae: *Microgale*), with new insights into geographic variation and distributional constraints. Biological Journal of the Linnean Society 83:1–22.

Olson, M. E., and A. Arroyo-Santos. 2009. Thinking in continua: beyond the "adaptive radiation" metaphor. BioEssays 31:1337–1346.

Otto-Bliesner, B. L., S. J. Marshall, J. T. Overpeck, G. H. Miller, A. Hu, and CAPE Last Interglacial Project members. 2006. Simulating arctic climate warmth and icefield retreat in the last interglacial. Science 311:1751–1753.

Outlaw, D. C., and G. Voelker. 2008. Pliocene climatic change in insular Southeast Asia as an engine of diversification in *Ficedula* flycatchers. Journal of Biogeography 35:739–752.

Ozawa, A., T. Tagami, E. L. Listanco, C. B. Arpa, and M. Sudo. 2004. Initiation and propagation of subduction along the Philippine Trench: evidence from the temporal and spatial distribution of volcanoes. Journal of Asian Earth Sciences 23:105–111.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Pearson R. G., C. J. Raxworthy, M. Nakamura, and A. T. Peterson. 2007. Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. Journal of Biogeography 34:102–117.

Pesole, G., C. Gissi, A. De Chirico, and C. Saccone. 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. Journal of Molecular Evolution 48:427–434.

Peterson, A. T., D. R. Stockwell, and D. A. Kluza. 2002. Distributional prediction based on ecological niche modeling of primary occurrence data. Pages 617–623 *in* J. Heglund and M. L. Morrison, eds. Predicting Species Occurrences: Issues of Scale and Accuracy. Island Press, Washington, D.C.

Peterson, A. T., J. Soberón, and V. Sánchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Phillimore, A. B., and T. D. Price. 2008. Density-dependent cladogenesis in birds. PLOS Biology 6:483–489.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

Phillips, S. J., and M. Dudík. 2008. Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. Ecography 21:161–175.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

Posada, D., and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53:793–808.

Price, T. D. 2008. Speciation in birds. Roberts and Company, Greenwood Village, Colorado.

Pritchard, J. R., and D. Schluter. 2001. Declining interspecific competition during character displacement: Summoning the ghost of competition past. Evolutionary Ecology Research 3:209–220.

Pybus, O. G., and A. Rambaut. 2002. GENIE: estimating demographic history from molecular phylogenies. Bioinformatics 18:1404–1405.
R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
Rabosky, D. L. 2006a. LASER: A maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. Evolutionary Bioinformatics Online 2006:257–260.
Rabosky, D. L. 2006b. Likelihood methods for detecting temporal shifts in diversification rates. Evolution 60:1152–1164.
Rabosky, D. L., and I. J. Lovette. 2008. Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? Evolution 62:1866–1875.
Rambaut, A. 1996. Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk/ [Accessed October 10, 2006].
Rambaut, A., and A. J. Drummond. 2007. Tracer v1.4. Available at: http://beast.bio.ed.ac.uk/Tracer/ [accessed 15 February 2010].
Rickart, E. A., L. R. Heaney, P. D. Heideman, R. B. Utzurrum. 1993. The distribution and ecology of mammals on Leyte, Biliran, and Maripipi islands, Philippines. Fieldiana: Zoology n.s. 72:1–62.
Roberts, T. E. 2006a. History, ocean channels, and distance determine phylogeographic patterns in three widespread Philippine fruit bats (Pteropodidae). Molecular Ecology 15:2183–2199.
Roberts, T. E. 2006b. Multiple levels of allopatric divergence in the endemic Philippine fruit bat *Haplonycteris fischeri* (Pteropodidae). Biological Journal of the Linnnean Society 88:329–349.
Rohling, E. J., M. Fenton, F. J. Jorissen, G. Bertrand, G. Ganssen, and J. P. Caulet. 1998. Magnitude of sea level lowstands of the last 500,000 years. Nature 394:162–165.
Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.
Rosenblum, E. B., M. J. Hickerson, C. Moritz. 2007. A multilocus perspective on colonization accompanied by selection and gene flow. Evolution 61:2971–2985.
Rowe, K. C., M. L. Reno, D. M. Richmond, R. M. Adkins, S. J. Steppan. 2008. Pliocene colonization and adaptive radiations in Australia and New Guinea (Sahul): Multilocus systematics of the old endemic rodents (Muroidea: Murinae). Molecular Phylogenetics and Evolution 47:84–101.
Rozas, J., Rozas, R., 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.
Ruedi, M. 1995. Taxonomic revision of shrews of the genus *Crocidura* from the Sunda Shelf and Sulawesi with description of two new species (Mammalia, Soricidae). Zoological Journal of the Linnean Society 115:211–265.
Ruedi, M. 1996. Phylogenetic evolution and biogeography of Southeast Asian shrews (genus *Crocidura*: Soricidae). Biological Journal of the Linnean Society 58:197–219.
Ruedi, M., M. Auberson, and V. Savolainen. 1998. Biogeography of Sulawesian shrews: Testing for their origin with a parametric bootstrap on molecular data. Molecular Phylogenetics and Evolution 9:567–571.

Sajona, F. G., H. Bellon, R. C. Maury, M. Pubellier, R. D. Quebral, J. Cotten, F. E. Bayon, E. Pagado, P. Pamatian. 1997. Tertiary and Quaternary magmatism in Mindanao and Leyte (Philippines): geochronology, geochemistry, and tectonic setting. Journal of Asian Earth Sciences. 15:121–153.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, New York.

Schmitt, L. H., D. J. Kitchener, and R. A. How. 1995. A genetic perspective of mammalian variation and evolution in the Indonesian Archipelago: biogeographic correlates in the fruit bat genus *Cynopterus*. Evolution 49:399–412.

Seehausen, O. 2007. Evolution and ecological theory - Chance, historical contingency and ecological determinism jointly determine the rate of adaptive radiation. Heredity 99:361–363.

Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. Systematic Biology 51:492–508.

Shimodaira, H., and M. Hasegawa. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17:1246–1247.

Siddall, M., Rohling, E.J., Almogi-Labin, A., Hemleben, C., Meischner, D., Schmelzer, I., Smeed, D.A. 2003. Sea-level fluctuations during the last glacial cycle. Nature 423:853-858.

Siler, C. D., J. R. Oaks, J. A. Esselstyn, A. C. Diesmos, and R. M. Brown. 2010. Phylogeny and biogeography of Philippine bent-toed geckos (Gekkonidae: *Cyrtodactylus*) contradict a prevailing model of Pleistocene diversification. Molecular Phylogenetics and Evolution doi:10.1016/j.ympev.2010.01.027.

Simberloff, D. 1978. Using island biogeographic distributions to determine if colonization is stochastic. The American Naturalist 112:713–726.

Simpson, G. G. 1977. Too many lines: the limits of the Oriental and Australian zoogeographic regions. Proceedings of the American Philosophical Society 121:107–120.

Smith, A. T., and Y. Xie 2008. A guide to the mammals of China. Princeton University Press, Princeton, New Jersey.

Soberón, J. 2007. Grinnellian and Eltonian niches and geographic distributions of species. Ecology Letters 10:1115–1123.

Soberón, J., and A. T. Peterson. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. Biodiversity Informatics 2:1–10.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Steppan, S. J., C. Zawadzki, and L. R. Heaney. 2003. Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in an oceanic archipelago. Biological Journal of the Linnean Society 80:699–715.

Storch, G., Z. Qiu, and V. S. Zazhigin. 1998. Fossil history of shrews in Asia. Pages 93–120 *in* J. M. Wójcik and M. Wolson, eds. Evolution of shrews. Mammal Research Institute, Polish Academy of Sciences, Białowieża.

Sukumaran, J., and M. T. Holder. 2009. DendroPy phylogenetic computing library version 3.0.0 (http://packages.python.org/DendroPy/).

Swofford, D. L. 1999. PAUP*4.0. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

Voris, H. K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. Journal of Biogeography 27:1153–1167.
Wallace, A. R. 1860. On the zoological geography of the Malay Archipelago. Proceedings of the Linnean Society of London 4:172–184.
Wallace, A. R. 1902. Island life, 3rd edn. Macmillan & Co., Ltd., New York.
Warren, D. L., R. E. Glor, and M. Turelli. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883.
Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. The American Naturalist 156:145–155.
Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475–505.
Wiens, J. J. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. Evolution 58:193–197.
Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.
Wright, S. 1950. The genetical structure of populations. Nature 166:247–249.
Yang, T. F., T. Lee, C-H. Chen, S-N Cheng, U. Knittel, R. S. Punongbayan, and A. R. Rasdas. 1996. A double island arc between Taiwan and Luzon: consequence of ridge subduction. Tectonophysics 258:85–101.
Yumul, G., C. Dimalanta, K. Queaño, and E. Marquez. 2009. Philippines, geology. Pages 732–738 in R. Gillespie and D. Clague, eds. Encyclopedia of Islands. University of California Press, Berkeley.
Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 ma to present. Science 292:686–693.

Appendix I

The following samples were used in analyses described in Chapter 1. Museum catalog numbers are given for specimens we used to generate new sequence data. Voucher prefixes are as follows: KU (University of Kansas Biodiversity Research Center), FMNH (Field Museum of Natural History), CMC (Cincinnati Museum Center), ROM (Royal Ontario Museum), MVZ (University of California at Berkeley, Museum of Vertebrate Zoology), USNM (United States National Museum), NK (Museum of Southwestern Biology, University of New Mexico), MSU (Mindanao State University, Iligan, Philippines), and IZEA (University of Lausanne). Approximate collection localities are given for samples sequenced herein; site numbers refer to Philippine collection localities in Fig. 3. Where sequence data were obtained from GenBank, we give GenBank accession numbers (those beginning with AB, AF, AY, or DQ) in place of voucher numbers.

Crocidura attenuata—**Hunan, China**: ROM 114916; **Guangxi, China**: ROM 116033.
Crocidura batakorum—**Site 29**: KU 165320; **Site 30**: KU 165412–165413, 165415, 165417–165422.
Crocidura beatus—**Site 18**: KU 165742, 165744–165746, 165748–165754; **Site 19**: KU 165756–165763, 165766–165768, 165775; **Site 20**: KU 165703–165708, 165710–165718, 165720; **Site 21**: FMNH 191345; **Site 22**: MSU no number available; **Site 23**: FMNH 146965–146966; **Site 24**: FMNH 147819, 166459; **Site 25**: CMC 1402, 1437; **Site 26**: CMC 1254, 1269, 1281, 1288, 1311; **Site 27**: FMNH 186804, 190154; **Site 28**: CMC 1719.
Crocidura beccarii—AF030496.
Crocidura brunnea—**Java**: ROM 101935.
Crocidura cf. *tanakae*—**Vietnam**: MVZ 185237, ROM 107661, 111293, 111317; **Hunan, China**: ROM 114960, 115004, 115005, 115021; **Guangxi, China**: ROM 116114, 116366, 116426, 116432, 116443.
Crocidura dsinezumi—AB077273.
Crocidura elongata—AF030507.
Crocidura foetida—**Borneo**: USNM 590298–590299, 590458.
Crocidura fuliginosa—**Peninsular Malaysia**: IZEA 3553, 3753, AB175079.
Crocidura grayi—**Site 1**: KU 164020–164021; **Site 2**: FMNH 185796–185797; **Site 3**: FMNH 167217–167221,175371–175373, 175376–175378; **Site 4**: FMNH 193407–193408, 193420, 193424–193425, 193429–193433, 193445–193447, 193850; **Site 5**: FMNH 188223–188227; **Site 6**: FMNH 186718–186719; **Site 7**: FMNH 183449–183453, 183457, 183466–183467, CMC 339; **Site 8**: FMNH 190702–190705; **Site 9**: FMNH 183468–183469; **Site 10**: USNM 573364, 573367, 573371, 573601–573602 (tissues for this series are held at FMNH); **Site 11**: FMNH 194718–194720; **Site 12**: KU 165176–165179, 165518; **Site 13**: CMC 1066; **Site 14**: KU 164433–164443.
Crocidura horsfieldii—AB175078.
Crocidura kurodai—AB175086.
Crocidura lasiura—AB077072.
Crocidura lea—AF030509.
Crocidura lepidura—**Sumatra**: MVZ 192172, 192174.
Crocidura levicula—AF030508.
Crocidura malayana—DQ630381.
Crocidura maxi—**Sumatra**: MVZ 192178.
Crocidura mindorus—**Site 13**: CMC 3582; **Site 15**: FMNH 145685–145686, 146788.
Crocidura musseri—**Sulawesi**: IZEA 4398, 4403.
Crocidura negrina—**Site 17**: KU 165046–165049, 165101–165108.
Crocidura nigripes—**Sulawesi**: IZEA 4382, 4400.
Crocidura orientalis—**Java**: ROM 101934.
Crocidura panayensis—**Site 16**: KU 164874–164878, 164992–164993.
Crocidura palawanensis—**Site 30**: KU 165463, FMNH 195214–195221, 195223–195224, 195227–195231, 195233, 195991–195996.
Crocidura paradoxura—AF030504.
Crocidura russula—AY918383.
Crocidura shantungensis—**Taiwan**: MVZ 181203.
Crocidura sp.—**India**: NK 10645.
Crocidura sp. 1—**Sulawesi**: NK 103507.
Crocidura sp. 2—**Sulawesi**: NK 103528.

Crocidura sp. 3—**Sulawesi**: NK 104104.
Crocidura rhoditis—AF030506.
Crocidura sibirica—AY994389.
Crocidura watasei—AB077074.
Crocidura wuchihensis—**Guangxi, China**: ROM 116090, 116095, 116129.
Suncus murinus—**Site 1**: KU 164724; **Site 16**: KU 164974; **Site 17**: KU 165125.

Appendix II

Samples used in Chapter 3.

Museum acronyms are as follows:
AMCC = Ambrose Monell Cryo Collection, American Museum of Natural History
KU = University of Kansas Biodiversity Research Center
ROM = Royal Ontario Museum
CMC = Cincinnati Museum Center
FMNH = Field Museum of Natural History
USNM = United States National Museum, Smithsonian Institute
IZEA = University of Lausanne
NK = Museum of Southwestern Biology
NTU = National Taiwan University
MVZ = Museum of Vertebrate Zoology, University of California, Berkeley
UAM = University of Alaska Museum

The "Phylogeny" and "Network" columns indicate whether or not the specimen was included in phylogenetic estimates and the statistical parsimony network, respectively.

Taxon	Catalog Number	Locality	Longitude	Latitude	Phylogeny	Network
Crocidura attenuata	AMCC101492	Ha Giang, Vietnam	22.7575	104.8303	YES	NO
Crocidura attenuata	AMCC101493	Ha Giang, Vietnam	22.7575	104.8303	YES	NO
Crocidura attenuata	ROM114916	Hunan, China	26.4167	111.0333	YES	NO
Crocidura attenuata	ROM116033	Guangxi, China	23.1167	105.9667	YES	NO
Crocidura batakorum	KU165421	Palawan, Philippines	8.75030	117.68960	YES	NO
Crocidura beatus	CMC1719	Mindanao, Philippines	6.05000	124.75000	YES	NO
Crocidura beatus	FMNH146965	Mindanao, Philippines	8.18320	124.74160	YES	NO
Crocidura beatus	KU165751	Samar, Philippines	11.80250	125.29280	YES	NO
Crocidura brunnea	ROM101935	Java, Indonesia	-6.75	106.95	YES	NO
Crocidura cf. *tanakae*	AMCC110774	Ha Tinh, Vietnam	18.067	105.9667	YES	YES
Crocidura cf. *tanakae*	AMCC110775	Ha Tinh, Vietnam	18.067	105.9667	YES	YES
Crocidura cf. *tanakae*	MVZ185237	Tam Dao, Vietnam	21.45	105.63	NO	YES
Crocidura cf. *tanakae*	ROM107661	Tuyen Quang, Vietnam	22.3333	105.4167	NO	YES
Crocidura cf. *tanakae*	ROM111293	Quang Nam, Vietnam	15.2	108.033	NO	YES
Crocidura cf. *tanakae*	ROM111317	Quang Nam, Vietnam	15.2	108.033	NO	YES
Crocidura cf. *tanakae*	ROM114960	Hunan, China	28.4167	114.1167	NO	YES
Crocidura cf. *tanakae*	ROM115005	Hunan, China	28.4167	114.1167	NO	YES
Crocidura cf. *tanakae*	ROM115021	Hunan, China	28.4167	114.1167	NO	YES

Crocidura cf. *tanakae*	ROM116366	Guangxi, China	23.1167	105.9667	YES	YES
Crocidura cf. *tanakae*	ROM116426	Guangxi, China	21.8456	107.8888	NO	YES
Crocidura cf. *tanakae*	ROM116432	Guangxi, China	21.8456	107.8888	NO	YES
Crocidura cf. *tanakae*	ROM116443	Guangxi, China	21.8456	107.8888	NO	YES
Crocidura foetida	USNM590299	Sarawak, Malaysia	2.65333	113.05139	YES	NO
Crocidura fuliginosa	AMCC101526	Ha Giang, Vietnam	22.7575	104.8303	YES	NO
Crocidura fuliginosa	FMNH168656	Kampot, Cambodia	10.61667	104.05	YES	NO
Crocidura fuliginosa	IZEA3753	Cameron Highlands, Malaysia	4.50000	101.55000	YES	NO
Crocidura grayi	FMNH167219	Luzon, Philippines	17.45833	121.06833	YES	NO
Crocidura grayi	USNM573367	Luzon, Philippines	13.66667	123.36667	YES	NO
Crocidura grayi halconus	KU164433	Mindoro, Philippines	12.83403	120.93188	YES	NO
Crocidura kurodai	NTU980	Taiwan	23.66800	120.98870	YES	NO
Crocidura kurodai	NTU981	Taiwan	23.66800	120.98870	YES	NO
Crocidura kurodai	NTU985	Taiwan	23.66800	120.98870	YES	NO
Crocidura lepidura	MVZ192172	Sumatra, Indonesia	3.566	98.1012	YES	NO
Crocidura maxi	MVZ192178	Sumatra, Indonesia	3.566	98.1012	YES	NO
Crocidura mindorus	CMC3582	Mindoro, Philippines	13.28000	121.01167	YES	NO
Crocidura mindorus	FMNH145685	Sibuyan, Philippines	12.45000	122.55000	YES	NO
Crocidura musseri	IZEA4398	Sulawesi, Indonesia	-1.26667	120.25000	YES	NO
Crocidura negrina	KU165103	Negros, Philippines	9.25856	123.17813	YES	NO
Crocidura nigripes	IZEA4400	Sulawesi, Indonesia	-1.26667	120.25000	YES	NO
Crocidura orientalis	ROM101934	Java, Indonesia	-6.75	106.95	YES	NO
Crocidura palawanensis	KU165463	Palawan, Philippines	8.75030	117.68960	YES	NO
Crocidura panayensis	KU164875	Panay, Philippines	10.81248	122.18153	YES	NO
Crocidura shantungensis	MVZ181203	Taiwan	24.1667	120.6333	YES	NO
Crocidura sp.	NK10645	India	18.36670	82.85000	YES	NO
Crocidura sp. 1	NK103507	Sulawesi, Indonesia	-2.93611	119.69732	YES	NO
Crocidura sp. 2	NK103528	Sulawesi, Indonesia	-2.93611	119.69732	YES	NO
Crocidura sp. 3	NK104104	Sulawesi,	-2.93118	119.71228	YES	NO

		Indonesia				
Crocidura sp. 4	UAM85085	Mondulkiri, Cambodia	12.13715	106.92142	YES	NO
Crocidura sp. 4	UAM85086	Mondulkiri, Cambodia	12.13715	106.92142	YES	NO
Crocidura sp. 4	UAM85087	Mondulkiri, Cambodia	12.13715	106.92142	YES	NO
Crocidura sp. 4	UAM85088	Mondulkiri, Cambodia	12.13715	106.92142	YES	NO
Crocidura sp. 4	UAM85089	Mondulkiri, Cambodia	12.13715	106.92142	YES	NO
Crocidura tanakae	KU165843	Batan, Philippines	20.47	121.991	YES	YES
Crocidura tanakae	KU165844	Batan, Philippines	20.47	121.991	YES	YES
Crocidura tanakae	KU165845	Batan, Philippines	20.47	121.991	YES	YES
Crocidura tanakae	KU165846	Sabtang, Philippines	20.285	121.871	YES	YES
Crocidura tanakae	KU165847	Batan, Philippines	20.383	121.934	YES	YES
Crocidura tanakae	KU165848	Batan, Philippines	20.383	121.934	YES	YES
Crocidura tanakae	NTU788	Taiwan	25.01700	121.01930	YES	YES
Crocidura tanakae	NTU969	Taiwan	24.17780	120.61020	YES	YES
Crocidura tanakae	NTU970	Taiwan	24.20500	120.53900	YES	YES
Crocidura tanakae	NTU971	Taiwan	24.17780	120.61020	YES	YES
Crocidura tanakae	NTU979	Taiwan	23.66800	120.98870	YES	YES
Crocidura wuchihensis	AMCC101499	Ha Giang, Vietnam	22.7575	104.8303	YES	NO
Crocidura wuchihensis	AMCC101508	Ha Giang, Vietnam	22.7575	104.8303	YES	NO
Crocidura wuchihensis	ROM116090	Guangxi, China	23.1167	105.9667	YES	NO
Suncus murinus	KU164724	Dalupiri, Philippines	19.085	121.241	YES	NO
Suncus murinus	KU164974	Panay, Philippines	10.8125	122.1815	YES	NO
Suncus murinus	KU165125	Negros, Philippines	9.2586	123.1781	YES	NO